Improving drug delivery to the lungs

Rottier, Bart Louis

Bart L. Rottier

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Towards better inhalation therapy

IMPROVING DRUG DELIVERY TO THE LUNGS

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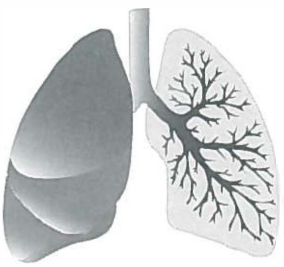


Bart L. Rottier

Stellingen behorende bij het proefschrift:

Improving drug delivery to the lungs
Towards better inhalation therapy

1 Inhaleren van geneesmiddelen via een inhalator met een hoge interne weerstand kan leiden tot een betere longdepositie dan met dezelfde inademingsnelheid inhaleren via een inhalator met een lage interne weerstand. (dit proefschrift)

2 Ook bij optimale omstandigheden die gericht zijn op het bereiken van de perifere luchtwegen, is een concentratieverschil van minimaal een factor 25 met de hogere luchtwegen ten nadele van de perifere luchtwegen onvermijdelijk. (dit proefschrift)

3 In vitro onderzoek van de combinaties van geneesmiddel en toedieningsvorm is belangrijk voor het beoordelen van de in vivo resultaten. (dit proefschrift)

4 Een mindmap is een goede manier om de samenhang van studies in een proefschrift duidelijk te maken. (dit proefschrift)

5 Het is het beste om dosisaerosol-voorzetkamer combinaties te gebruiken in een ruimte met een hoge relatieve luchtvochtigheid. (dit proefschrift)

6 Communicatie is volgens het CanMEDS model een van de zeven essentiële competenties in de opleiding tot medisch specialist en deze competentie is ook onmisbaar in wetenschappelijke communicatie. (dit proefschrift)

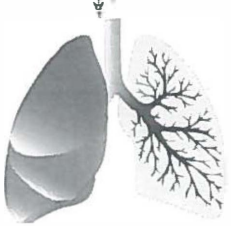
7 Onder de grootste druk komen de mooiste diamanten tot stand.
(Maarten van der Weijden op Twitter)

8 Het feit dat een dure placebo pil krachtiger werkt dan een goedkope wijst op het grote belang van positieve suggestie als vorm van geneeskunst in de geneeskunde.
(Waber RL et al. Commercial features of placebo and therapeutic efficacy. *JAMA* 2008; 299(9):1016-7)

9 Het betalen van een faire prijs (Fair Trade, Fair Wear) voor producten kan tot een eerlijkere verdeling van welvaart leiden en ontwikkelingshulp deels overbodig maken.

10 Het enorme aantal metingen met laserdiffractie maakt dat het essay " Why most published research findings are false" (Ioannidis J, *PLoS Med* 2005; 2(8): e124) op deze studies niet van toepassing is.

11 Look for the bare necessities, the simple bare necessities, forget about your worries and your strife. (Baloo in the Junglebook movie)



Bart L. Rottier, november 2012

About the cover:

Prof. dr. Jan Kimpen offered the author of this thesis a picture of another piece of art by Joan Miró on the occasion of graduating as a paediatrician. That piece of art was colourful, but with less detail. "The singing fish" symbolizes getting the big picture and paying attention to the details. Aerosol particles with different sizes, shapes and colours can be seen. "The singing fish" expresses a colourful life full of passion.

Cover: "The singing fish", by Joan Miró (Barcelona, 1893-1983)
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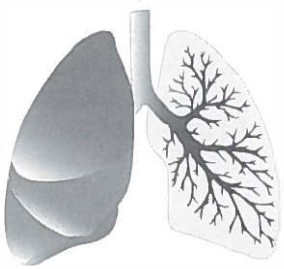

RIJKSUNIVERSITEIT GRONINGEN

Improving drug delivery to the lungs

Towards better inhalation therapy

Proefschrift

ter verkrijging van het doctoraat in de
Medische Wetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
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in het openbaar te verdedigen op
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door

Bart Louis Rottier

geboren op 6 augustus 1965

te Amsterdam

Promotor:	Prof. dr. E.J. Duiverman
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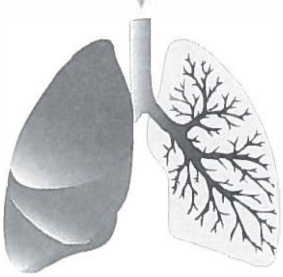
Beoordelingscommissie:	Prof. dr. D.S. Postma Prof. dr. P. Colombo Prof. dr. R.M.F. Berger

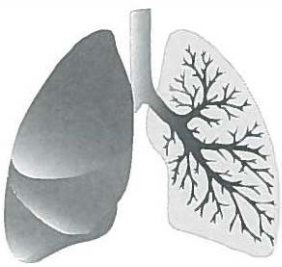
To all who teach

pap en mam
Andrea, Maarten, Pepijn en Sebastiaan

Contents



CHAPTER 1



Introduction and aim

1.1 General principles of inhalation therapy

Drug inhalation is the cornerstone in the treatment of pulmonary diseases like asthma and chronic obstructive pulmonary disease (COPD). Treating these diseases by specific targeting of the airways with bronchodilating and anti-inflammatory drugs has resulted in major improvements to control these diseases. Asthma and COPD are highly prevalent diseases and cause considerable morbidity and mortality. Therefore, many patients would potentially benefit from improved inhalation devices or a better use of existing devices. Besides asthma and COPD, other less prevalent diseases may (partially) be treated with aerosol therapy such as cystic fibrosis (CF), chronic lung disease (CLD or bronchopulmonary dysplasia BPD), primary ciliary dyskinesia (PCD) and incidentally bronchiolitis in infancy, laryngotracheobronchitis, non-CF bronchiectasis, alpha-1-antitrypsine deficiency (www.clinicaltrials.gov) and pulmonary hypertension.[1-5] Tuberculosis and lungcancer may be indications for inhaled therapy in the near future. Tuberculosis is a clear and present danger with an estimated 1.7 million deaths each year and more than 9 million new cases.[2,3] In this contagious disease, inhaled drug treatment could target alveolar macrophages that harbour TB bacilli, and lead to high drug concentrations in lung tissue as well as systemic delivery.[4,5] Lung cancer is a prevalent disease, for which the combination of high local doses and few systemic effects of inhaled chemotherapy would be a great advantage as would be the inhalation of immunosuppressants after lung transplantation.[6-8]

Next to inhaled treatment of pulmonary diseases, inhaled therapy to provide systemic drug delivery via the lung may open the way for treatment of systemic disorders like diabetes mellitus with insulin, or delivering vaccines (e.g. influenza, measles).

Many drugs have been registered for inhalation or are under development *(Table 1.1)*.

However, inhaled therapy is not that simple. Drug deposition in the lung after inhalation therapy is highly variable and ranges from 1-50% of the label claim depending on a variety of factors such as the inhalation device, delivered drug mass, inhalation manoevre (technique, inspiratory flow rate and breath hold), adherence to treatment , disease related and environmental factors.[9-12] Improvements in lung deposition may therefore, potentially increase treatment efficacy, reduce costs and save time spent on treatment, thereby possibly increasing adherence to treatment.

Although inhalation therapy has many potential advantages, there is considerable room for improvement of drug delivery to the lungs. The general aim of this thesis is to investigate possible improvements in inhalation treatments for pulmonary and non-pulmonary diseases.

Drug class	Examples
Antibiotics	aztreonam, ciprofloxacin, colistin, gentamicin, tobramycin
Anticholinergics	ipratropriumbromide, tiotropriumbromide, aclidiniumbromide
Antifungals	amfotericin B, ambisome, pentamidine
Antivirals	ribaverin, zanamavir
Anti mycobacterial	isoniazid, streptomycine
Bronchodilators/$ß_2$ agonists	salbutamol, terbutalin, salmeterol, formoterol
Chemotherapy	gemcitabine, doxorubicin, cisplatin
Gene transfer	Cystic Fibrosis Transmembrane Regulator (CFTR)
Inhaled corticosteroids	beclomethasone/beclomethasone extra fine, budesonide, ciclesonide, flunisolide, fluticasone, mometasone, triamcinolone
Immunosupressants	cyclosporine
Lungproteins	alfa-1-antitrypsine, surfactant protein
Mucolytics	acetylcysteine, DNAse, hypertonic saline, mannitol
Muscle relaxants	Magnesiumsulphate
Osmotic agents	hypertonic saline, mannitol
Pulmonary vasodilators	iloprost
Systemic	insulin, L-DOPA, vaccines, morphine, fentanyl, dihydroergotamine, interferon beta

Table 1.1 *: examples of inhaled drug treatment*

Advantages of inhaled drugs

A key advantage of drug delivery by inhalation for pulmonary diseases is that it enables delivery of a high dose of aerolized drugs to the target area with considerably less systemic side effects. Therefore the dose used by inhalation can be up to a factor 10 lower than an intravenous or oral dose. This favorable benefit-harm ratio can be illustrated by the side effects of $ß_2$–agonists, which have no or only mild side effects when inhaled, but important side effects as tachycardia and agitation when administered orally or intravenously.[13] In the same way, inhaled corticosteroids and antibiotics also have less side effects compared with systemically distributed drugs. Further, some drugs such as DNAse or hypertonic saline can only be administered by inhalation. Advantages of treating systemic diseases with aerolized medicine are that this route is feasible for drugs that are poorly absorbed orally or degrade by first-pass losses in the liver.[14] Another advantage of inhalation therapy is the potential reduction in the use of injections (vaccines, insulin) and, depending on the formulation, the absence of a need for keeping drugs refrigerated, which would be a huge advantage in many developing countries with lower costs and easier logistics.
Finally, aerosol drug delivery has a rapid onset of action for both local and systemic effects compared to orally taken drugs. Particular for drugs like $ß_2$ –agonists this advantage is of great importance.

Disadvantages of inhalation therapy

Despite the advantages of high local concentrations with low systemic effects, inhalation therapy in daily practice is challenging due to a flawed inhalation technique by many individuals using this form of treatment. Inhalation manoeuvres may be time consuming (especially when drugs are to be nebuliszed

or when multiple doses are required), which may reduce treatment adherence. Many different inhalation devices are available and most of them require different inhalation manoeuvres to obtain an optimal result. Repeated instruction is required,[15] but in spite of that, many devices are still widely used incorrectly because their precise principle of operation is unknown to the patient and healthcare workers. In clinical trials, adherence to medication, is estimated to be around 70%, but mean adherence rates clearly decline with increased dosing frequency.[16] Inhalation devices that are more easy to use might therefore probably improve adherence.

The bronchial tree

The bronchial tree is a branching system starting with the trachea (generation 0) which divides into a main stem bronchus for each lung (generation 1) and then continues to branch by dichotomy like a tree, thereby progressively reducing the airway diameters *(Figure 1.1)*.[17] Bronchi and bronchioles are conducting structures whose main function is to distribute the air into the peripheral units and are therefore called the "conducting airways". Non-respiratory bronchioles are transitional elements and form the "transitional zone" taking the air into the respiratory bronchioles, alveolar ducts and alveoli which comprise the "respiratory zone" where gas exchange takes place. This model of branching results in an exponentially increasing cross sectional area for airflow and therefore, resistance and air velocity decrease towards the alveoli. The relatively large conducting airways (generations 1-11) contribute to less than 1% of the total surface area of the lung, the transitional airways (generations 12-16) add only 4% and the airway generations 17 to 23 (the respiratory airways), contribute for more than 95% to the total airway surface area.[18]

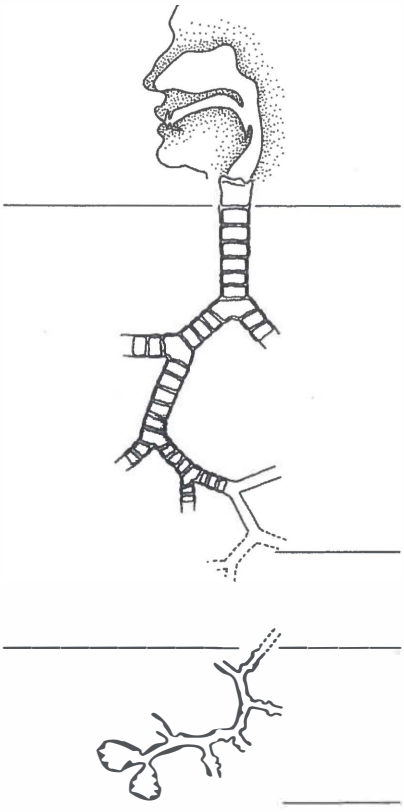


Figure 1.1 *The branching airways with exponentially increasing surface area towards the smaller airways.*

Deposition mechanisms in the airways

Particles travelling with the air flow through the respiratory tract are subjected to different forces. When a particle has the same velocity and flow direction as the surrounding streamlines of the air, only the force of gravity (F_G) is relevant. This force (F_G = m.g, in which m is the particle mass and g the acceleration of gravity) causes the particle to fall (settle). When the particle enters a bifurcation where the streamlines of the airstream change direction, particles trajectories may deviate from these air streamlines. Particles with higher inertia (mass) than air molecules tend to continue travelling in the original direction. This creates a friction force (resistance) with the air molecules flowing around the particles, expressed as (Stokes') drag (or resistance) force: $F_D = 3.\pi.\eta.U_{PA}.D$, in which η is the viscosity of the air, U_{PA} is the air velocity relative to the particle and D is the particle diameter. This drag force changes the flow direction of the particle towards that of the surrounding air and reduces the velocity in the original flow direction. The distance over which the particle velocity in the original direction is reduced to zero by the resistance force is called the stopping distance (S). This stopping distance is a function of particle momentum, which is the product of initial particle velocity (U_0) and particle mass (m). The higher the initial particle velocity and/or particle mass are, the longer the stopping distance. Whether a particle travels into the next airway generation thus depends on the velocity and mass of the particle and the airway diameter. The two mechanisms of deposition that are related to these forces and the particle stopping distance are inertial impaction and sedimentation. A third mechanism of deposition is diffusion *(Figure 1.2)*.

Inertial impaction is the deposition of aerosol particles on the walls of an airway conduit by a high momentum. This is either due to high velocity, high particle mass or both. Inertial impaction is the predominant way of deposition in the upper respiratory tract (conducting airways), where the air (particle) velocity is still relatively high and particles of all sizes (including the largest ones) enter.

Sedimentation is particle settling by the force of gravity. Sedimentation mainly occurs in the central and peripheral airways, where the air velocity is much lower and distances to the airway wall are shorter. As soon as the drag (resistance) force (F_D) and gravity (F_G) are in equilibrium, a stationary settling velocity for the particle is achieved. Sedimentation is a time dependent mechanism of deposition and the efficacy of particle settling by sedimentation may thus be increased by a longer residence time of particles in the airways by a breathhold.

Diffusion is movement of particles by collision with air molecules. Significant travelling distances by diffusion are only achieved for particles smaller than 0.1 micron. Since particles of this small size hardly carry any drug mass, diffusion does not contribute relevantly to lung deposition.

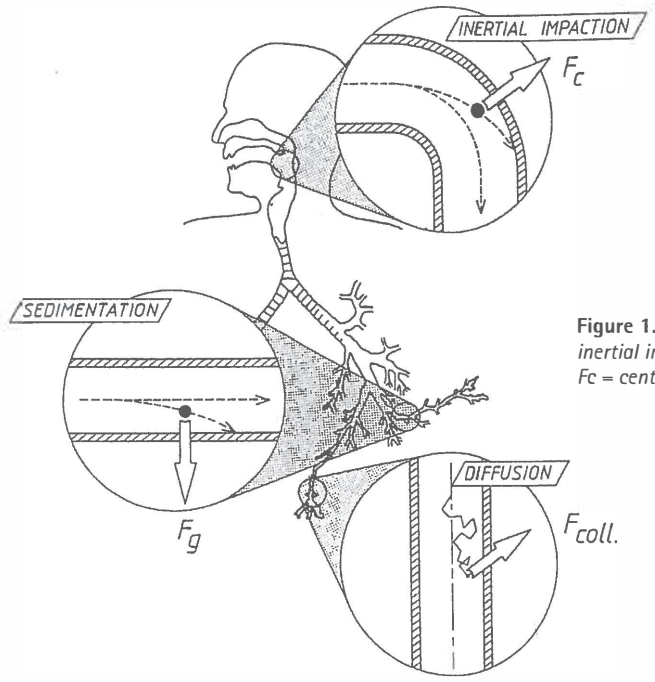


Figure 1.2 *Deposition mechanisms in the airways: inertial impaction, sedimentation and diffusion Fc = centrifugal force, Fg =gravitational force*

How to define aerosol particles

Size is one of the key determinants of the behaviour of each particle and the amount of drug it carries. It is therefore essential to know the particle size distribution of an aerosol in order to make a prediction of the amount of drug that can be deposited in large, intermediate or small airways. When particles are not round (spherical) but are irregular of shape, e.g. most solid particles from a dry powder device, a prediction of the behaviour in the airway is possible by determining the aerodynamic diameter. The aerodynamic diameter takes both size, shape and density into account. Some large particles, such as large porous particles as used for the Exubera insulin formulation and the Tobramycin Inhalation Powder (TIP) may be very light despite their size and are therefore more likely to follow the airstream into the smaller airways than to impact in the larger airways. Thus, large size but low density particles may have the same aerodynamic behaviour as smaller particles with higher density, i.e. these particles have the same aerodynamic diameter.

The definition of aerodynamic diameter D_A is the diameter of a round droplet with the density of water (unit density , 1 g/cm^3) having the same terminal settling velocity in still air as the particle considered. Particles with the same aerodynamic diameter all have the same stopping distance, the same settling velocity and experience the same drag force. The volume of an irregularly shaped particle can also be expressed as an equivalent volume diameter D_E of a round (sphere) particle. The aerodynamic diameter D_A results from the equivalent volume diameter D_E when additionally taking the dynamic shape factor (χ) and particle density (ρ) into account.

$$D_A = D_E\sqrt{(\rho/\chi)}$$

The dynamic shape factor (χ) is the ratio of the actual resistance force on the irregularly shaped particle to the resistance force on a round particle having the same volume (D_E) and the same velocity relative to the air. Both particle density ρ and the dynamic shape factor χ vary from 1 to 1.5 for solid (non-porous) particles, which results in aerodynamic diameter D_A varying in a range from 0.82 to 1.22 times D_E.

As an example, the volume of an irregularly shaped particle with a dynamic shape factor (χ) = 1.17 and particle density (ρ) = 1.50 g/cm^3 leads to an aerodynamic diameter $D_A = 1.14D_E$ *(Figure 1.3)*.

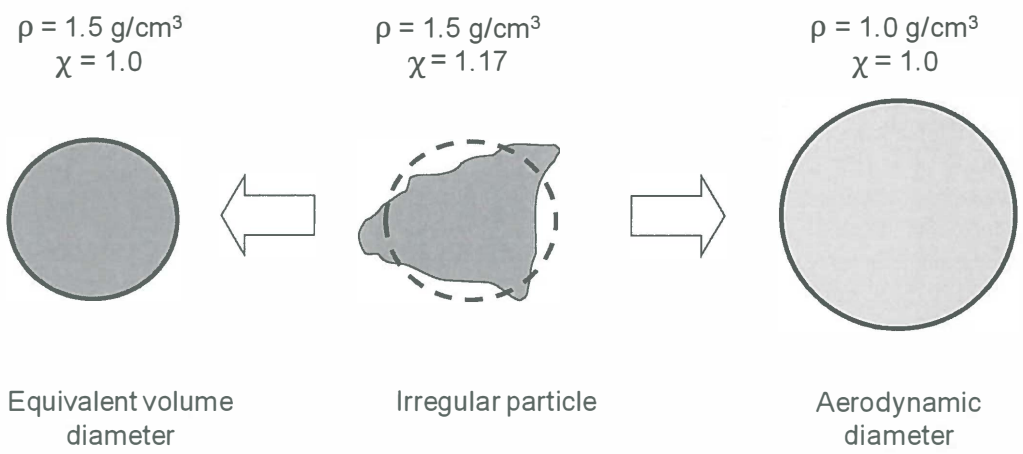


Figure 1.3 *Methods to characterise irregularly shaped particles in the aerosol technology. DE is the equivalent volume diameter: the diameter of a sphere (round particle) having the same volume as the irregular particle in consideration. DA is the aerodynamic diameter, defined as the diameter of a sphere (c = 1) with the density of water (r = 1 g/cm3) having the same settling velocity in still air. Particles with the same DA have the same aerodynamic behaviour.*

A mathematical model, used by the International Commission on Radiological Protection predicts that particles < 5 µm (aerodynamic diameter) are most appropriate for deposition in the lung.[19] In patients, it has been shown that inhalation of particles within the aerodynamic diameter range 1-3 µm and inhaled with a flow rate of 30 L/min to total lung capacity followed by a 3-5 second breath hold, results in the best distribution over the whole lung.[20] Particles smaller than1µm (aerodynamically) are largely exhaled after inhalation; particles larger than 3-5 µm impact predominantly in the upper airways. A combination of a specific aerodynamic diameter and inhalation manoeuvre therefore determines whether particles deposit mainly in the conducting, transitional of peripheral airways. The effect of particle size may be different in patients with obstructive airways compared to healthy controls. Total lung deposition of monodisperse 1µm particles increased in subjects with airway obstruction (smokers, patients with asthma and COPD) in proportion to the severity of obstruction as measured with FEV_1.[21] This may also be true in children as their airway diameters are smaller than adults, especially when compromised by disease. Inflammation, mucus hypersecretion and bronchoconstriction cause a reduction of airway diameter and result in enhanced flow velocity and turbulence, which in turn increases deposition in the airways.

Aerosol generation devices

Aerosol generation devices can be divided into four different groups depending on their principle of operation: pressurized metered dose inhalers (pMDIs), dry powder inhalers (DPIs) nebulizers and soft mist inhalers.[22-25] Auxiliary equipment to these devices is available to increase ease of operation or to improve lung deposition such as spacers or valved holding chambers, breath triggered dose release mechanisms for pMDI's, computer controlled breathing and breathing pattern adapted aerosol delivery for nebulizers and soft mist inhalers.

Pressurized metered dose inhalers (pMDIs)

pMDIs have a pressurized reservoir chamber holding the drug in solution or suspension. The drug is released from a metering chamber with a fixed volume. The dose release is actuated manually and a good press and breathe (hand-lung) coordination is needed to inhale the drug into the peripheral airways. With the phasing out of chlorofluorocarbon (CFC) propellants, completed in 2013, and the switch to hydrofluoroalkane (HFA) propellants, certain aerosol characteristics changed. Particularly the plume released by an HFA-pMDI may have a lower velocity and a higher temperature because of less propellant evaporation, especially if ethanol is added as co-solvent for the drug. For adults who might still directly inhale from the pMDI, the lower plume velocity reduces mouth and throat deposition whereas the higher plume temperature reduces the so called 'cold freon effect' that makes patients sometimes stop inhaling. Moreover, beclomethasone in HFA (with ethanol co-solvent) changed to a smaller median particle size, whereas fluticasone (without co-solvent) did not, which may have implications for area of deposition. Children need to use pMDIs with a valved holding chamber (VHC, also referred to as spacers), as press – breath coordination cannot be expected to be adequate. The use of a VHC can reduce mouth and throat deposition as the aerosol is not released with a high plume velocity from the spacer. Furthermore, the cold freon effect is eliminated as the aerosol temperature rapidly increases within the VHC and there is no need to coordinate actuation with inhalation. A VHC has a one way valve that allows inspiration through the chamber only; the exhaled breath is diverted and does not enter the VHC .
Valved holding chambers either have a face mask, which should have a close fit, or a mouthpiece. As the nose is built as an efficient filter to protect the lungs, as soon as children can control breathing through their mouth, a mouthpiece should be used. The lower aerosol plume velocity and higher temperature from HFA inhalers compared to CFC inhalers may respectively lead to less impaction in the spacer and less condensation of water on aerosol particles.

Breath actuated pMDIs are a special type of pMDIs which release a drug dose triggered by an inspiratory flow rate of 30 L/min (Autohaler®) or 20 L/min (Easibreathe® also called Redihaler®). The air flow switches a valve when the trigger flow rate is achieved and this enables a preloaded spring to open the metering chamber and to release the dose.

Dry Powder Inhalers (DPIs)

DPIs are devices that deliver medication to the lungs as a dry powder aerosol.[26] Most DPIs with β_2 –agonists and corticosteroids contain micronized drug blended with larger (lactose) carrier particles which dilutes the drug and improves the flow properties of the powder. Dilution and improvement of the flow characteristics are needed for reproducible drug dosing. Because the micronised drug particles adhere to the surface of the carrier crystals, the stability of the powder is high (no segregation) but the carrier-drug agglomerates are too large to be inhaled. Release of the drug particles from the carrier crystals during inhalation is needed. This process is called dispersion or de-agglomeration. The energy for dispersion is

derived from the inhaled air stream; this energy generates forces to break-up the adhesive forces between drug and carrier particles. As the kinetic energy increases with increasing air flow rate through the DPI, the fraction of released drug particles may increase with the flow rate as well. The drug particles are carried deep into the lungs, while the larger carrier particles deposit mainly in the oropharynx.

Nebulizers

Current nebulizers convert drug solutions or suspensions into an aerosol by either a jet stream generated by a compressor (a nebulizer-compressor system) or by means of high frequency (ultra sonic) vibration applied to the surface of the drug solution. In the Netherlands ultra sonic nebulizers are scarcely used and therefore, this type of nebulizer is not being discussed.

A jet nebulizer basically consists of a nebulizer cup with the drug solution or drug suspension) connected to a so-called two-fluid nozzle. The two-fluid nozzle consists mostly of two tubes (capillaries). One tube starts in the nebulizer cup (to transport the drug solution), the second tube is connected to a pressure system. The pressurised air (jet flow) causes a negative pressure and this creates suction and as a consequence the liquid starts to rise in the capillary. As soon as the liquid has reached the end of the tube, the liquid surface of the drug solution is disrupted by turbulence from the jet stream and small droplets are formed. An impact body (baffle) is placed over the two-fluid nozzle and larger droplets impact against the baffle and fall back in the nebulizer cup. Only the smallest particles within the aerodynamic size range appropriate for inhalation can pass the baffle *(Figure 1.4)*. This mechanism results in relatively long nebulization times.

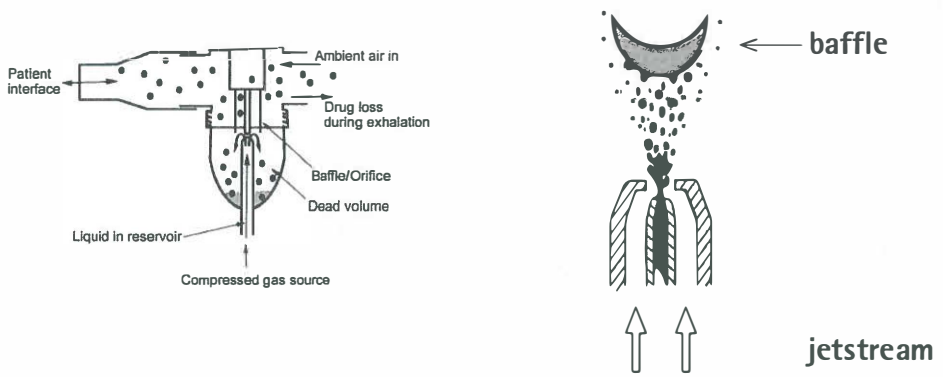


Figure 1.4 *Conventional jet nebulizer (left) with jetstream flow and baffle in more detail (right).*

Conventional jet nebulizers produce a constant flow of aerosol containing air, resulting in losses of aerosol in the environment during periods of exhalation.
Open vent nebulizers (e.g. Sidestream) continuously entrain air into the nebulizer cup via a special vent. Breath assisted open vent nebulizers only take air into the nebulizer cup with inhalation (e.g. Pari LC® Plus, Ventstream®). During exhalation, the inlet vent closes and an outlet vent in the exhaust tube opens to discharge the used air. When the patient does not inhale, both vents are closed so no drug is lost into the environment.
Breath actuated nebulizers (e.g. AeroEclipse®) build up pressure in the nebulizer cup during periods of exhalation which depresses the drug solution in the capillary of the two-fluid nozzle. As a result

aerosol generation can only take place during inspiration - thereby also eliminating drug waste during exhalation.

New developments in nebulizer therapy

A new development in nebulizer therapy is the introduction of a system controlling the supply of air to the nebulizer depending on the phase of the inspiratory manoeuvre (eg Akita® Jet with Pari LC®Sprint). A plain air bolus can be given before the aerolized drug is released, to prevent the drug of reaching the alveolar region, or following the inhalation of the drug an air bolus can push the drugs into obstructed airways.

Other developments have focussed on shortening the nebulization time by using mesh technology. Mesh nebulizers either have a vibrating perforated membrane (mesh) in contact with the drug solution, or a vibrating piston in the nebulization cup. The oscillation of the mesh (or piston) forces the drug solution through funnel shaped pores in the membrane. Mesh nebulizers are efficient, quiet and they can be battery operated. Examples are the eFlow®, eFlow® rapid and I-neb. The eFlow® has a very low residual volume to reduce drug waste, whereas the eFlow® rapid has a large residual volume so it can be filled with the same fill volume as classic jet nebulizers. The large residual volume thus compensates for the more efficient nebulization. The I-neb has a so called Adaptive Aerosol Delivery (AAD), adapting the release of the next aerosol based on the previous three inhalations, during the first 50-80 % of the inhalation. This improves drug transport into the peripheral airways and it reduces nebulization time and drug use significantly. The latter is especially important for the nebulization of expensive drugs such as iloprost for pulmonary hypertension. The I-neb also incorporates software to monitor adherence. Its metering chamber can deliver a pre-set volume ranging from 0.25 to 1.4 mL depending on the drug solution chosen, with a residual volume of about 0.1 mL. Nebulizers that only nebulize during (part of) the inspiration are also called smart nebulizers.

A different principle of aerosol generation has been used for the Respimat® Soft Mist™Inhaler. The Respimat aerosol generating system takes position in between a pMDI and a classic jet nebulizer.
It produces an aerosol from an aqueous drug solution by impaction of two fluid jets.[25]

How to characterise inhalation aerosols?

Size is one of the key determinants for the aerodynamic behaviour of aerosol particles and for the amount of drug an aerosol particle may carry. For aerosolized solutions, the volume of the droplet and thus the amount of drug carried is proportional to the third power of the radius. Small droplets may be numerous, but carry little drug; e.g. droplets with a 2.5 µm radius (a 5 µm droplet) carry 125 times the amount of drug compared to a droplet with a 0.5 µm radius (a 1 µm droplet). The mass distribution as function of the aerodynamic particle diameter for an aerosol can predict the amount of drug that can be deposited in large, intermediate or small airways.
Particle size distributions of aerosols can be measured with cascade impactor analysis (CIA) or laser diffraction analysis (LDA).[27] Cascade impactors have been considered the gold standard for inhaler testing as they classify mass fractions of the drug dose into usually 7 (4-9) aerodynamic size classes. The aerosol from the inhaler is drawn through the impactor system with a constant flow rate and forced to undergo a series of turns in the airstream. In subsequent stages, each consisting of a nozzle and a collection plate, particles with a momentum that is too high to follow the streamlines of air from the nozzle will impact on the plate underneath that nozzle *(Figure 1.5)*. By increasing the air and thus particle velocity in subsequent nozzles, classification occurs towards finer particles in subsequent stages. Cascade impactor

analysis is the method of choice to assess the aerodynamic particle size distribution from all inhalation devices.

The results from cascade impactor analysis can be processed into cumulative mass distribution curves as function of the aerodynamic diameter. From those distribution curves the fine particle fractions (FPFs) or Mass Median Aerodynamic Diameters (MMADs) can be derived. Many clinicians emphasize the importance of the MMAD; however the MMAD is a mean value and provides no information on the particle size distribution. Aerosols with the same MMAD may thus not be comparable. Further, MMAD does not give information about the fraction of the dose that has been collected in the impactor. Drug retentions in a nebulizer system or losses of drug in a spacer may reduce the amount of drug of which the MMAD is measured to a fraction of the label claim. DPIs usually contain a drug-carrier mixture. The fraction of drug particles released from the carrier particles during inhalation is generally less than 50%. This may lead to an aerosol cloud with a MMAD < 5 µm. The remaining carrier-drug mixture is emitted with a larger MMAD, mainly determined by the particle size distribution of the carrier.

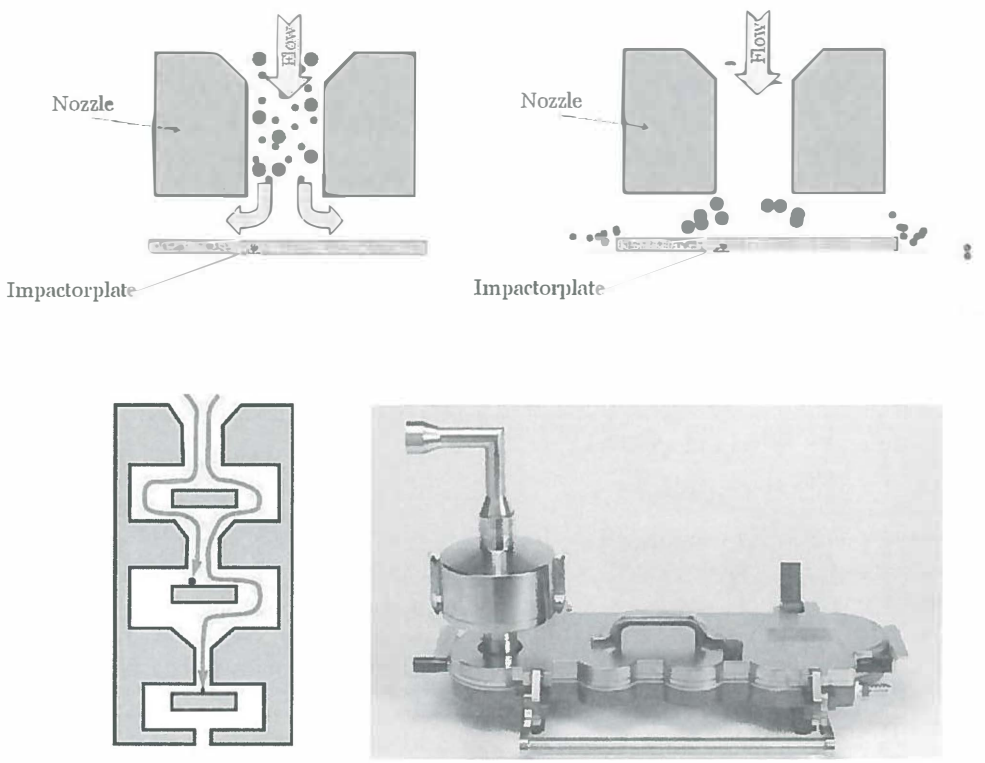


Figure 1.5 *The principle of cascade impaction analysis (CIA). Only the smallest particles pass at each level (upper figures, left and right and lower figure left. Low right: the Next Generation Impactor (NGI).*

An aerosol from a DPI thus consists of two different drug fractions, each with their own particle size distribution. Knowledge of the fine particle dose (FPD) is more relevant for the amount of drugs that can

be expected to be deposited in the airways than a single MMAD value. The FPD can be expressed as the mass of drug in particles within a certain aerodynamic diameter range. The fine particle 'fraction' (FPF) is the fine particle dose expressed as a percentage from either the label claim or the delivered dose to the impactor. The delivered dose to the impactor should then also be given and preferably be related to the label claim.

Disadvantages of cascade impactor analysis are that the procedure is time consuming and the distribution of the aerosol over a series of different stages makes it difficult to measure single aerosol doses as these may be too small to be analysed accurately. Therefore, usually a larger number (5-10) of aerosol doses is analysed and this analysis results in a mean value for the FDP. Further, in cascade impactor analysis the number of classes relevant to inhalation (1-5 μm) is relatively small. Laser diffraction analysis (LDA) is a fast, sensitive and highly reproducible alternative. Particles are passed through a laser beam and the light diffracts at different angles depending on the size and optical properties of the particles *(Figure 1.6)*. The diffracted light is collected on a series of concentric detector rings and the complex diffraction pattern is processed into a volume distribution as function of the particle diameter, assuming that these particles are spherical. Disadvantages from laser diffraction analysis are that the data interpretation is more difficult than that for data from cascade impactor analysis, as no aerodynamic diameters are measured. Laser diffraction analysis yields a volume median diameter (VMD or X_{50}; X_{10} and X_{90} define the range). X_{10}, X_{50} and X_{90} are derived from the cumulative volume distribution *(Figure 1.7)* with 10, 50 and 90% of the volume in particles smaller than those values.
For aerosol particles with the same density (e.g. spherical droplets from pMDIs) with the same density (irrespective of size, e.g. spherical droplets from pMDIs), the VMD measured by laser diffraction analysis equals the mass median diameter. If the aerosol cloud consists of a low concentration of an aqueous solution or homogeneous suspension, the VMD even equals MMAD (as measured with cascade impactor analysis) as the dynamic shape factor (χ) and particle density (ρ) both equal 1. For non spherical particles, as from DPIs, laser diffraction analysis can be used to measure dispersion efficacy by comparison with the primary particle size as a reference.

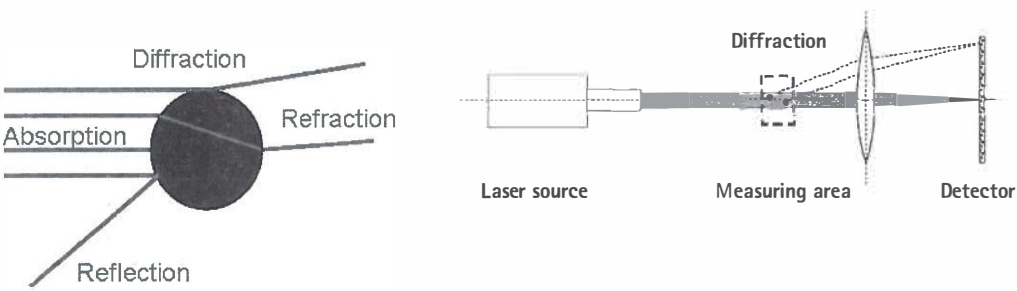


Figure 1.6 ***(upper left).*** *Diffraction of lightwaves around a small particle* ***B (upper right).*** *Particles of the same size are projected on the same part of the detector by a special lens (Fourier lens).*

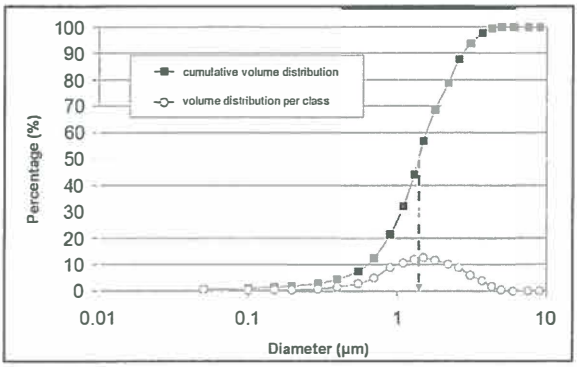


Figure 1.7 ***A (left).*** *Laser diffraction analysis (LDA) adaptor.* ***B (right)*** *Cumulative volume distribution (closed squares) volume frequency distribution as function of the particle diameter (closed circles).* X_{50} *or volume median diameter (VMD) is pointed out by the arrow.*

The relevance of the inspiratory flow manoeuvre

The way patients inhale has an effect on aerosol generation from a DPI, both on total delivered dose and on the Fine Particle Fraction. Further, the inhalation manoeuvre also affects the site of deposition in the airways and the fraction of drug deposited. If particles enter the respiratory tract with a high travelling speed, they will impact in the upper airways. High flow rates should thus be avoided when the lower airways are the target area.

The effect of flow rate on the site of deposition increases with increasing particle diameter *(Figure 1.8).* In a study with monodisperse particles of three different sizes, losses in the oropharynx are substantial at flow rates higher than 30 L/min, particularly for 3 to 6 micron particles.[20] On the other hand, small particles need a relatively long period of residence time to deposit by sedimentation. The particle's settling velocity decreases with the square of the diameter. For particles of 1 micron, the time needed to travel a distance of 0.25 mm is almost 8.5 s. For particles in the aerodynamic size range between 1 and 3 micron this takes considerably less time. Therefore a breathhold pause following deep inhalation of at least 5 and preferably 10s is necessary.

Thus, the higher the inspiratory flow, the lower the deposition in the peripheral airways and these data plea for a low inspiratory flow rate. However, the energy within the inhaled air stream is also used for aerosol generation from DPIs. A low inspiratory flow rate may therefore lead to a relative lack of energy for effective powder dispersion. Ineffective powder dispersion in turn leads to larger particles in the aerosol and a lower fine particle dose. To sum it up, the inspiratory flow should be low enough to allow for peripheral deposition, and high enough to disperse the powder adequately and form the optimal FPD.

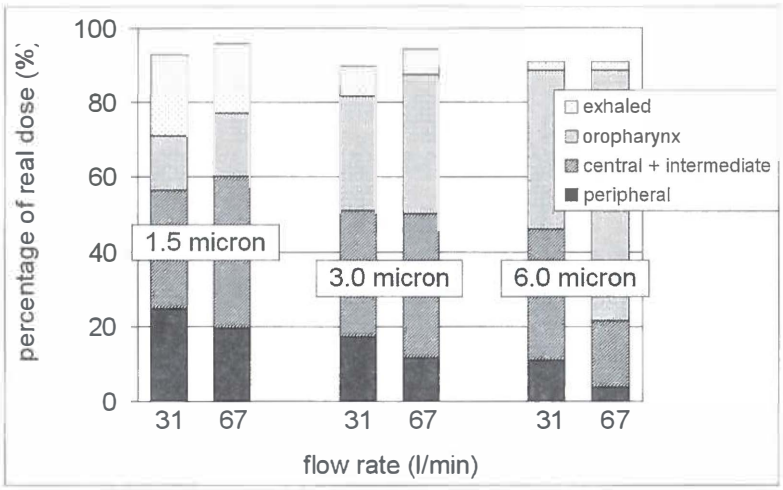


Figure 1.8 *Deposition depends on particle size and inspiratory flow, derived from Usmani.*[30]

The relevance of inhaler resistance

A DPI can be considered as a resistance to air flow. The flow rate affects the performance of dry powder inhalers (effective powder dispersion), but its resistance determines the attainable range of flow rates through the device. Importantly, it is not the absolute flow rate that determines the fine particle dose from a dry powder inhaler, but the resulting kinetic energy, which also depends on the design of the inhaler. Effective inhalers convert the kinetic energy of the air flow into powder dispersion forces. High local air velocities within the inhaler are required for this, rather than a high air flow rate through the inhaler. The way to achieve high local velocities without a high inspiratory flow rate is to design narrow, tortuous channels, turbulent zones or whirl chambers, which all contribute to a higher air flow resistance of the inhaler. Therefore, the prerequisite for an effective DPI is to achieve a sufficient pressure drop across the inhaler rather than to achieve a high flow rate through the device. Current DPIs all perform well at a pressure drop between 2 and 4 kPa.[28] These DPIs do have different air flow resistances though, and as a consequence the flow rate over the same 4 kPa pressure drop across the DPI may vary from 45 to 110 L/min. This will result in different deposition patterns for the same aerosol particles *(Figure 1.8)*, with the highest peripheral deposition fractions at 2 to 4 kPa obtained for the inhalers with the highest air flow resistance, leading to the lowest flow rates.

The driving force for an air flow (Φ) through the inhaler is a pressure drop (dP) across the inhaler, as obtained during inhalation through the inhaler. For DPIs, there is a linear relationship between the square root of the pressure drop across the inhaler and the flow rate: $\sqrt{dP} = \Phi . R$, in which R represents the air flow resistance. For a given pressure drop dP, the flow rate (Φ) thus increases with decreasing air flow resistance (R).

A high flow rate can only be maintained for a short period of time. Although patients with a lower vital capacity generate lower pressure drops than healthy volunteers across the same air flow resistance, they can generate higher pressure drops across higher resistances than across low resistance inhalers.

A too forceful inhalation will result in an increase in oropharyngeal deposition *(Figure 1.8)*. Aditionally, deposition in the lung shifts towards larger airways. If DPIs can compensate for these shifts towards larger airways and oropharynx at higher flow rates with an increased fine particle dose, the drug dose delivered to the smaller, peripheral airways may remain the same. In spite of what is often claimed, DPIs that release an increased fine particle dose with higher flow rates are therefore to be preferred above DPIs that deliver a flow independent fine particle dose. Turbuhaler®, Novolizer® and Easyhaler® are examples of inhalers with an increase in delivered fine particle dose with increasing flow rate.

Message in a bottle

This principle of a pressure drop across lower and higher air flow resistances can be demonstrated with a test inhaler with exchangeable air flow resistance, connected to an empty polyethylene terephthalate (PET) bottle as a manometer *(Figure 1.9)*. When used with the high air flow resistance, a pressure difference across the air flow resistance can be obtained and the water bottle crumples completely. The high air flow resistance results from a small orifice and the resulting flow rate is low. The time to achieve pressure equilibrium between the lungs and the bottle is then longer. When the air flow resistance is low, because of a larger orifice diameter, the attainable pressure drop across the test inhaler with the same inspiratory effort is much lower and the time to reach pressure equilibrium is then shorter than with the high resistance. This reflects in less crumpling of the water bottle despite a shorter inhalation.

Besides an appropriate flow rate and a breath hold after maximal deep inhalation, exhalation to residual volume prior to inhalation is important to target the peripheral lung. This deep exhalation prior to inhalation is important to optimize sufficient refreshment of the alveolar volume.

Figure 1.9 *This principle of a pressure drop across lower and higher air flow resistances can be demonstrated with a test inhaler with exchangeable air flow resistance, connected to an empty polyethylene terephthalate (PET) bottle as a manometer. When used with the high air flow resistance, a high pressure difference across the air flow resistance can be obtained and the water bottle crumples completely. The high air flow resistance results from a small orifice through which only a low flow rate is possible. The time to achieve pressure equilibrium between the lungs and the bottle is therefore relatively long. When the air flow resistance is low, because of a larger orifice diameter, the flow rate (at the same inspiratory effort) is much higher. Therefore, the time to reach pressure equilibrium on both sides of the the orifice is shorter than with the high resistance and the attainable pressure drop across the test inhaler with the same inspiratory effort is much lower. This reflects in less crumpling of the water bottle despite a shorter inhalation*

Drug distribution and drug concentration in the airways

The surface area of the airways increases from less than 1% of total airway surface area for airway generations 0-11 to less than 5% for airway generations 12-16 and to more than 95% for the peripheral airways, generations 17-23 *(Figure 1.1)*. Even with an optimal inhalation manoeuvre of an aerosol with an optimal particle size distribution, the exponentially increasing airway surface area will clearly lead to a considerable decreasing drug concentration towards the alveoli.

1.2 Asthma and COPD

Astma is a chronic inflammatory disease with reversible airway obstruction based on mucosal oedema, increased mucus production and bronchoconstriction. Asthma is clinically characterized by episodes of wheezing, dyspnoea and/or cough. Bronchoconstriction and bronchodilator reversibility can be demonstrated with pulmonary function tests. The pathophysiology of asthma includes chronic, mostly eosinophilic, inflammation in both large and small airways *(Figure 1.10)*.[29,30]

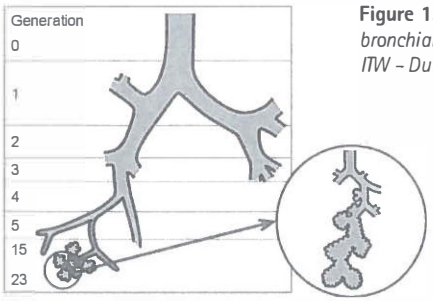


Figure 1.10 *The inflammatory processes in asthma involve the entire bronchial tree up to the alveolar region (presented with permission, ITW – Dutch Inhalation Technology Working Group).*

Being an inflammatory disease, anti-inflammatory treatment is the cornerstone of treatment and all guidelines recommend inhaled corticosteroids (ICS) as the first choice of maintenance anti-inflammatory treatment.[31,32] As the number of ICS receptors increase towards the lung periphery *(Figure 1.11)*[33] targeting the small airways has been called the new challenge of ICS treatment.[34]

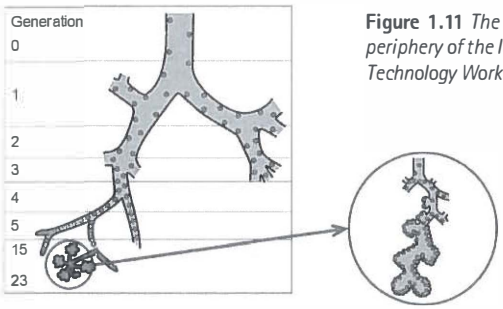


Figure 1.11 *The number of corticosteroid receptors increases towards the periphery of the lung (presented with permission, ITW – Dutch Inhalation Technology Working Group).*

ICS treatment with extrafine particles aims to treat the entire bronchial tree including the small airways better than larger particles would be able to do. Extrafine particles penetrate better into the small airways, which is expected to lead to a higher and more homogeneously distributed lung deposition than larger particles.[20] This might result in asthma control using lower daily doses with fewer side effects. Particle size may be of particular importance in young children because of their smaller airways and different breathing pattern, with higher breathing frequency and relatively small tidal volume. There are two ICS with extrafine particle sizes: extrafine (HFA) beclomethasone dipropionate (licensed in Europe for children 5 years of age and older) and ciclesonide (licensed for children 12 years of age and older). Ciclesonide, a pro-drug which is converted in the airways into the active metabolite des-CIC, is thought to have a reduced potential for local and systemic side effects. Due to its binding to protein and slow release from the protein, it is licensed for once daily use, which may improve adherence. Clinical studies in adults suggest that ICS with a large fine particle fraction (1-3 µm) might be more effective than ICS with larger particles.[35-37] A clinically important improvement in quality of life, measured with the Asthma Quality of Life Questionnaire, was observed at 12 months for HFA-beclomethasone vs CFC-beclomethasone in half the dose equivalent in daily life.[35] A study in the General Practice Research Database (GPRD) including more than 25% children aged 5-12 years (but children were not separately reported on), found that therapy with HFA-beclomethasone resulted in similar or better asthma control than with fluticasone at the same or lower prescribed dose.[36] Small ICS particles have been demonstrated to be superior to larger particles in improving forced expiratory volume in 1 second (FEV_1) [38] and bronchial hyperreactivity as measured with metacholine.[39]

Episodes of bronchoconstriction can be treated with short acting $ß_2$ –agonists, which are considered first choice rescue treatment. For asthma not controlled by ICS only, adding long acting $ß_2$ –agonist as maintenance treatment is an option.[31,32]

Chronic obstructive pulmonary disease (COPD) is an inflammatory disease in which the whole respiratory tract is affected, from the central to the so called small airways of less than 2 mm in internal diameter.[40] Patients with COPD suffer from symptoms resulting from air flow limitation. Treatment varies with COPD-severity as expressed in GOLD stage 1-4 from short acting bronchodilators as needed to long acting bronchodilators with or without ICS (www.goldcopd.org).

Preferably, devices which are used in asthma and COPD are pMDI´s with or without spacers, breath actuated pMDIs and DPIs. Nebulizers are only prescribed when there is a need for concomitant oxygen supply.
Administration of bronchodilators with a pMDI-spacer combination is as effective as administration with nebulizers as was shown in children with asthma[41,42], adults with asthma[42] and adults with mild exacerbations from COPD.[43,44]
Magnesium sulphate by inhalation has been investigated as an alternative to intravenous magnesium sulphate in the treatment of acute asthma exacerbations.[45] The mechanism of action is smooth muscle relaxation which is additive to the effect of conventional bronchodilators.
A trend in improvements in pulmonary function and a decrease in hospital admissions compared to placebo did not reach statistical significance and currently this treatment is not recommended.

1.3 Cystic Fibrosis

CF is a rare disease with decreased life expectancy due to end stage respiratory failure.
The pathophysiology of CF is based on a defect in the CFTR gene and the resulting chloride and other ion channel defects result in abnormally thick airway secretions. Impaired mucociliary clearance leads to further retention of mucus, allowing bacterial colonization, infection and subsequent neutrophilic inflammation. The resulting airway damage leads to bronchiectasis. Patients with CF in general have to inhale more different drugs as they grow older.

Inhaled therapy in patients with CF is targeted towards *Pseudomonas aeruginosa (P. Aeruginosa),* the resulting inflammation and mucus plugs which cause obstruction. A European consensus for inhaled medication and inhalation devices for lung disease in CF is available.[46]

Inhaled antibiotic treatment in CF started off with low dose gentamycin.[47] The second inhaled antibiotic was tobramycin, used for *Pseudomonas aeruginosa* infection. Tobramycin is used for eradication when a *P. Aeruginosa* is isolated for the first time or for maintenance treatment when patients are chronically colonized with this micro-organism.[48] Also, colistin can be used for maintenance treatment when persistent colonization is present. The current eradication standard for *P. Aeruginosa* is inhalation of tobramycin 300 mg twice daily for 4 weeks or inhaled colistin 2 dd 80-160 mg for 3 months combined with ciprofloxacin for 3 weeks.[46] Next to colistin and tobramycin, azthreonam has become available for inhalation (AZLI or Cayston).[49]
The most recent development in inhalation of antibiotics in CF, is a tobramycin inhalation powder (TIP), which is licensed for maintenance therapy, but not for eradication.[50,51] TIP has to be inhaled twice daily and during each dosing 4 capsules of 28 mg of tobramycin should be inhaled. Each capsule needs to be inhaled twice to minimize retention. Since TIP is very hygroscopic the inhaler is replaced weekly. Other inhaled antibiotics in CF under development are fosfomicin/tobramicin and ciprofloxacin.[52,53]

Particularly in CF, targeting the drugs to the whole lung may be challenging as the aerosols are more likely to deposit in better ventilated, healthier areas of the lung, whereas delivery to more severely affected regions is needed. Secretions in the airway will result in a more turbulent flow pattern and in increased central airways deposition. The target area for inhaled antibiotics in CF has not been clearly described.

Besides infection, the exaggerated immune response in the CF airways has been taken responsible for ongoing respiratory deterioration in CF. This inflammatory response is often treated with ICS, although there are only limited data supporting this.[54] ICS are also used in patients with CF and concomitant asthma, although it is difficult to diagnose asthma in CF patients. Symptoms and lung function abnormalities may be similar. Third, high dose ICS are used in CF patients with allergic bronchopulmonary aspergillosis (ABPA).

Sputum from CF patients contains 3-5 times higher DNA levels than in non-CF individuals because of decayed inflammatory cells and bacteria. This makes the sputum more viscous. rhDNase is a drug that cleaves neutrophil derived DNA, making the sputum more fluid and therefore has beneficial effects in CF. Its efficacy has been demonstrated in patients from the age of 6 years onwards and a reduced exacerbation rate and a slight increase in pulmonary function has been demonstrated in patients using

DNase compared to placebo.[55] A 5-8% overall improvement in FEV_1 in patients using DNase has been shown, although individual response may differ considerably, ranging from marked improvement to even deterioration of 20% in FEV_1. Guidelines advise to start from the age of 6 onwards for a three month trial period, regardless of the result of pulmonary function tests. In a study comparing DNase delivered from 2 different nebulizer-compressor systems as an aerosol with either an MMAD of 2.1 µm or an aerosol of 4.9 µm, the 2.1 µm aerosol resulted in a trend to a greater improvement in FEV_1, although this was not significant.[56] Small airways targeting with a smart nebulizer (Akita) had a more positive effect in adherent children compared to larger airway targeting also with Akita. The primary outcome for this study was forced expiratory flow at 75% of forced vital capacity (FEF(75%)). FEF(75%) increased significantly by 0.7 standard deviation (5.2% predicted) in the large airways group and 1.2 standard deviation (8.8% predicted) in the small airways group. Intention-to-treat analysis did not show a significant difference in treatment effect between groups.[57] Usually DNAse is dosed once daily, but a schedule of alternate days may be just as effective.[58]
Nebulized hypertonic saline (7%,) improves mucociliary clearance in patients with CF.[59] On a group level, DNAse is more effective than hypertonic saline, but there is variation in individual response.[58] Hypertonic saline can be used as a cheaper alternative or added to daily DNAse treatment for better clearance of the airways.[59]

Bronchodilators can be used for those CF-patients who show a relevant bronchodilatory response, i.e. a more than 9% increase in predicted FEV_1 after short acting beta-agonists.[60]

Other inhalation drugs that are used occasionally in CF or have been under study are other antibiotics (liposomal ciprofloxacin), antifungals (amphotericin-B, liposomal amphotericin-B), alternative Chloride channel activators (Denufosol), and viral and non-viral agents for gene transfer to the lung.[61,62]. Unfortunately, although promising in phase 2 clinical trials,[63] in phase 3 denufosol did reach up to its expectations and failed to show any effect on primary or secondary endpoints when compared to placebo.

Drugs in CF can be administered with pMDI-spacer combinations, breath actuated devices, DPIs and nebulizer systems. Nebulized drugs in CF are registered with a fixed nebulizer combination and the use of unregistered combinations is discouraged. Tobramycin for inhalation solution (TOBI®) is licensed for inhalation with a PARI LC® Plus nebulizer and a DeVilbiss® Pulmo-Aide® compressor. A second inhaled tobramycin solution (Bramitob®), is also registered with the PARI LC® Plus nebulizer, but together with a PARI TURBO Boy® compressor. Aztreonam inhalation solution (Cayston®) is licensed for use with the eFlow® nebulizer. If particle size distribution and output would be equivalent with other than the registered systems, in vivo equivalence could be anticipated and at least guide the clinician in making educated decisions regarding off label drug-device combinations.
Seven drugs currently in the drug development pipeline for CF will need to be administered by inhalation (http://www.cff./research/DrugDevelopmentPipiline/)

1.4 Systemic diseases treated with inhaled drugs and vaccines

The lung has a large alveolar surface area, which is highly perfused and would therefore be an excellent way of administrating drugs to the systemic circulation. In the alveolar region, the barrier for absorption is thin and minimal mucociliairy clearance occurs. The lungs are even more permeable to small molecules than the gastrointestinal tract.[64] The optimal target area within the lungs for the delivery of drugs to the systemic circulation is therefore the alveolar region with an estimated resorption surface area of 90 to120 m^2 in women and men, respectively.[65] It is likely however, that systemic drug delivery can also partly take place by absorption via the small airways. In general, proteins with molecular weights between 6,000 and 50,000 Dalton are relatively resistant to most peptidases that are anchored in the plasma membranes of all cells and attack peptides at the ends of the amino acid chain, releasing one or two amino acids at a time. This results in good bioavailabilities following inhalation for drugs like vaccines, levo-dopa (CVT-301) or insulin.[64]

Peptides and small molecules that are or have been under research for systemic delivery include (but are not limited to)[14]: fentanyl to treat pain[66], dihydroergotamine for migraine[67] and interferon beta to treat Multiple Sclerosis. Further, inhaled heparin is being developed to inhibit thrombosis[68], as anti-inflammatory and anti-coagulant agent for the treatment of smoke inhalation in burn victims[69] and inhaled heparin is also tested to treat Idiopathic Pulmonary Fibrosis.[70]

The advantages of inhaled dry powder vaccines, like influenza vaccine in case of an epidemic or pandemic outbrake of this disease, are that inhaled medicines are easier to distribute (no cold chain storage), easier to administer and thus reduce costs (especially important in low income countries). Moreover, vaccine delivery via the respiratory tract, alimentary tract, or skin might elicit mucosal immune responses on the site of virus entry and better cellular immunity, thus improving effectiveness.[71] Mucosal immunization might also prove additional advantage compared to the relative ineffective Mycobacterium bovis Bacillus Calmette-Guérin (BCG) vaccination; a spray-dried BCG allows efficient aerosol delivery of TB vaccines targeted deep into the lung.[72] Respiratory administration of measles vaccine proved to be as effective as subcutaneous administration and is adaptable to mass campaigns, thereby avoiding the risks associated with injections. Measles vaccination by aerosol therefore could help measles eradication.[73]

Inhaled insulin has been considered the most promising drug to be used on a large scale for systemic delivery but has only shortly been available because of many shortcomings in the devices used.[74-76]

1.5 Aims of this thesis

The main objective of this thesis is to improve treatment of respiratory and systemic diseases by improving inhalation treatment. The conditions for adequate pulmonary drug delivery based on physical laws have been discussed above.

First, current anti-inflammatory treatment in asthma is reviewed *(chapter 2)*. Clinical effects of ICS depend on delivered dose and particle size distribution of the aerosol. A high fraction of particles 1-3 µm seems to be important as the inflammatory process in asthma involves all airways. Therefore in *chapter 3* the particle

size distribution and delivered doses from commonly used ICS pMDI's combinations were determined. pMDIs are almost always used in combination with valved holding chambers or spacers. Spacers decrease the dose available for inhalation due to losses in the spacer, but the amount of decrease is unknown for all current ICS-spacer combinations. Therefore, to determine the best pMDI-spacer combination and define environmental conditions that result in the highest fine particle output, we determined interdevice variation and the impact of air humidity and inspiratory flow rate on both spacer output and the particle size distribution *(chapter 4)*. The desired target area, best device and inhalation manoeuvre to treat *Pseudomonas aeruginosa* in the CF airway have not been clearly described. The possible answers to those gaps in current knowledge are given in *chapter 5*. New nebulizers became adopted by the CF community before registration studies were performed. The hypothesis that the vulnerable mesh technology may lead to a decreased output and a change of particle size distribution in daily life compared to using a registered drug-nebulizer combination was tested in *chapter 6*. We determined the effect of inspiratory flow rate on output and particle size of newly registered tobramycin and colistin preparations in registered and unregistered drug-nebulizer combinations. If particle size distribution and output would be equivalent to the registered drug-device systems, in vivo equivalence could be anticipated and at least guide the clinician in making evidence based decisions regarding off label drug-device combinations *(chapter 7)*. Systemic delivery of drugs is possible if drugs can be targeted to the distal lung region where absorption occurs. Inhalation of insulin was one of the most promising examples. However, despite the potential advantages of inhaled insulin, e.g. no cold chain storage and no need for needles, the first insulin inhaler (Exubera®, Pfizer) was withdrawn from the market after only one year. We hypothesized that the used inhaler was too inefficient, large and expensive to serve its purpose. Therefore we aimed to show that improved inhalation technology eliminates these drawbacks whereas systemic insulin availability per mg inhaled insulin can be doubled *(chapter 8)* which may also serve as a proof of concept for other systemic inhaled medication.

A summary of the main findings and the implications for clinical practice are given in *chapter 9*, together with a description of common myths in inhalation technology, and directions for future research.

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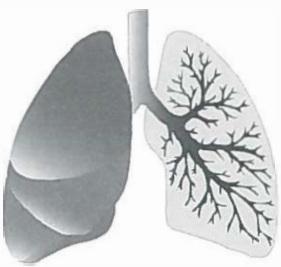
CHAPTER 2



Anti-inflammatory drug therapy in asthma

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Eric J. Duiverman

Paediatr Respir Rev. 2009;10: 214-9

Abstract

Asthma is a disease with chronic inflammation of the airways and anti-inflammatory treatment is a logical treatment. Inhaled corticosteroids (ICS) remain the cornerstone of anti-inflammatory therapy in recent international guidelines. Asthma cannot be cured by any medication: if the drug is discontinued, the disease manifestations return. This has been proven at all ages. In preschool children the diagnosis of asthma is difficult to establish. In this heterogeneous group ICS or leukotriene receptor antagonists (LTRA) are just as effective as placebo; in the future it will hopefully be possible to describe characteristics of responders. LTRA are an alternative in mild asthma, especially when mono-triggered viral related wheeze is present. Theophylline is effective and also has bronchodilatory properties, which need to be balanced against the relatively frequent side effects. The working mechanisms of anti-inflammatory asthma medications including ICS, LTRA, cromones, macrolides and theophylline are described.

INTRODUCTION

Asthma is characterised by bronchial hyper-reactivity, chronic symptoms with intermittent attacks of cough and wheeze, skewing of pulmonary T cells to a Th2 phenotype and increased airway eosinophils defined as a chronic eosinophilic inflammation of the airways.[1,2] Anti-inflammatory maintenance therapy remains the cornerstone of asthma treatment in this era.[3-5] Inhaled corticosteroids are considered to be the most effective anti-inflammatory treatment, safe in appropriate doses when given to asthmatic children and adults, even as long term therapy.[3-5] Actual points of debate include: (1) The use and indications of ICS in preschool wheezing children; (2) How to monitor the use of ICS based on symptoms, lung function, bronchial responsiveness measurement, induced sputum or by means of non-invasive exhaled NO measurement; and (3) Should ICS be prescribed in asymptomatic asthmatic children during puberty and adolescence? This review will focus on indications, efficacy, monitoring and side effects of anti-inflammatory therapy in children with asthma.

INHALED CORTICOSTEROIDS (ICS)

Guidelines advise maintenance therapy with ICS for asthmatic children with any of the following features: using inhaled β_2 agonists three times a week or more; being symptomatic three times a week or more; or waking one night a week. In addition, ICS are recommended for consideration in adults and children aged 5-12 years who have had an exacerbation of asthma requiring oral corticosteroids in the last two years.[4,5] Corticosteroids diffuse across cell membranes, bind to glucocorticoid receptors (GRs) in the cytoplasm, transfer to the nucleus where the receptor-corticosteroid complex binds to DNA eventually leading to changes in gene transcription. The desired effect is inhibition of synthesis of inflammatory proteins, but the systemic side effects of corticosteroids are also caused by changes in gene transcription.[6] For many years it was suggested that moderate to higher dosages of ICS (400 to 1000 mcg of budesonide) proved to be effective as well as safe in children. Even after long term therapy with moderate to high doses of ICS growth reduction seemed to be transient and final adult height proved to be normal. However, Visser et al [2004] have shown that moderate to high dosages ICS may have systemic side effects, serving as a reminder to all clinicians of the importance of minimising corticosteroid exposure in growing children.[7]

The ICS dose-response curve is rather steep in most asthmatics, meaning that low doses are sufficient in most situations. This has led to the view that the lowest effective ICS dose should be found to achieve asthma control.[3-5] Former guidelines recommended a step down approach, which means that after a relatively higher ICS starting dose the optimal minimum dose should be determined.[8] Several months were needed before this therapeutic optimum was reached. Currently, this approach is no longer advised.[3-5] Based on clinical symptoms a starting dose is chosen, which in most cases would include a daily dose of 200-250 mg fluticasone propionate (FP) or 400 mg budesonide (BUD) / beclomethasone (BDP). If clinical remission has been reached after three months of treatment this starting dose should be lowered. If the child is not symptom free, the starting dose may be doubled, after issues like therapy adherence, adequate inhalation technique and smoking behaviour have been checked.[3-5] An alternative approach is the use of extra-fine beclomethasone or ciclesonide. Because of small particles these formula may give higher peripheral lung deposition, which may be more effective, and as a consequence lower ICS doses can be used (i.e. 200 mg daily instead of 400 mg BDP or BUD).[9,10] This may be an important advantage when treating very young children with small airway diametres. Ciclesonide is a promising new ICS which can be prescribed once daily and claims to be ineffective outside the lung.[11,12] It has been registered in many countries for adults and children over the age of six years.

ICS in preschool aged wheezing children

A recent ERS task force report reviewed the problem of wheezing in childhood in detail.[12] An emphasis on preschool wheezing reflected the recent changes in the characterisation of wheezing phenotypes which have occurred as a result of a number of longitudinal studies. Most reported wheeze occurs in acute, short-lived episodes, in association with viral (upper) respiratory infection and in the absence of interval symptoms.[13,14] About 40% of all preschool children regularly wheeze during common cold infections. About two-thirds of these children lose their symptoms and are symptom free after the age of six years. Studies of the efficacy of ICS in preschool children have given conflicting results. Some studies show that ICS are effective while others find no effect at all. The most plausible explanation for these differences is that there is no single wheezing phenotype in young children but several. If the diagnosis asthma can be stated with a high level of certainty level in preschool children, then a positive response to ICS may be expected. However, if the diagnosis is not clear or less likely, ICS are unlikely to be not useful. Schokker et al [2008] found no effect of ICS maintenance treatment in 2-5 years old children with recurrent wheeze for whom the general practitioner decided to prescribe ICS.[15] In this placebo controlled study with steroid naïve children, there was no difference between ICS maintenance therapy and symptomatic β_2 treatment, measured by symptom free days and nights or lung function assessed by forced oscillometry. From this, and other studies, it may be concluded that ICS are not indicated in most wheezy infants and young children. Many young children are prescribed ICS inappropriately. This is both a threat to the child with potential side effects as well as an economic burden. Several investigators have tried to define criteria based on which one may conclude whether a young child is asthmatic or not, but still it remains extremely difficult to reliably diagnose asthma at a young age.[12,15] Even if there is a felt to be a high likelihood of asthma and ICS therapy appears to be indicated, the question still arises as to whether such a child should be treated with ICS for a long period of time. Guilbert et al [2006] have shown that in preschool children at high risk for asthma, two years of ICS therapy did not change the development of asthma symptoms or lung function during a third, treatment-free year *(Figure 2.1)*.[16] In other words, ICS maintenance treatment failed to prevent the ultimate outcome of progression to asthma in this cohort of preschool children.

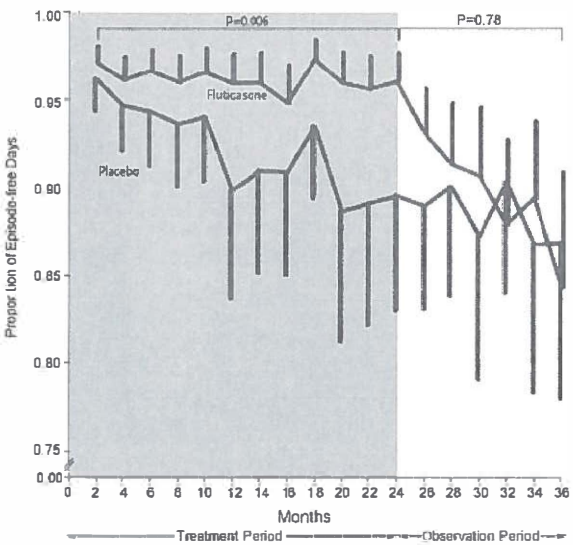
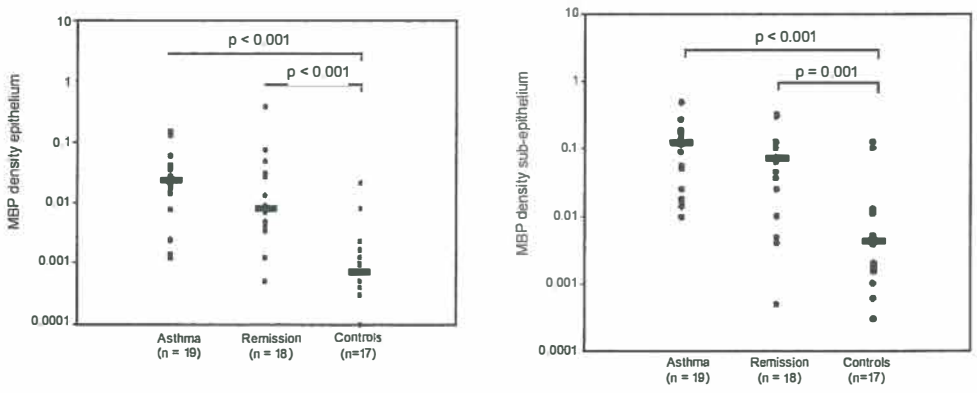


Figure 2.1 *In preschool children with a high risk for asthma, treatment with fluticasone 100 ug 2 daily (European label claim, equivalent to 88ug 2 daily US label claim) compared to placebo (the shadowed part of the figure) resulted in a significant increase in proportion of symptom free days during the two years of active treatment. Active treatment was then discontinued and the children were in observational follow up for another year. The difference in symptom free days gradually disappears. Anti-inflammatory treatment with ICS in preschool children at high risk of developing asthma takes care of symptoms but does not prevent the further development of asthma(reproduced with permission, Copyright © 2006 Massachusetts Medical Society. All rights reserved).*

ICS in puberty with respect to prognosis in adult life

Although ICS treatment is the most effective anti-inflammatory asthma therapy and has been prescribed for more than thirty years now, there are no data from literature that suggest that permanent remission can be reached and that airway remodelling can be prevented.[16-18] About half of asthmatic children lose their symptoms during puberty or adolescence, and maintenance treatment will be stopped either by themselves or on medical advice. Have these children really outgrown their asthma or is this symptom free period just temporary? Many "ex-asthmatics" will e symptomatic before the age of thirty years again. Van der Toorn et al. [2001] have shown that "ex-asthmatic children", who were symptom free for many years, and did not require any asthma medication, still had asthma characteristics when lung function was measured at the age of twenty years.[19] In addition, in this cohort the eosinophils in induced sputum and exhaled NO values were as high as in symptomatic asthmatics. In bronchial biopsies, basal membrane thickness in the asymptomatic group was at least intermediate between healthy controls and asthmatics, or comparable with symptomatic subjects *(Figure 2.2)*.[19] These data show that, although these young adults were asymptomatic for many years, they retained typical asthmatic characteristics, suggesting that they remain predisposed to developing symptoms of asthma in later life. In a later study, Van der Toorn et al. [2005] re-investigated the same patients in order to determine whether combination therapy with ICS and long acting ß2-agonist (fluticasone-salmeterol) lead to improvement in lung function in these "asymptomatic asthmatics" *(Figure 2.3)*.[20] These studies suggested that asymptomatic adolescents were still at risk, and should be treated with ICS maintenance although they seem to have outgrown their asthma. Whether this approach gives rise to a better prognosis for lung structure and function during later adult life has not been determined. Regardless of the potential merit of therapy, a major barrier remains in motivating asymptomatic adolescents or young adults to use medication.



Figuur 2.2 *Major Basic Protein (MBP) is an eosinohil related protein. The figure shows the MBP density in both epithelium (top panel) and sub-epithelium (lower panel) for three groups of study subjects: currently asthmatics (left), "ex-asthmatics"(adolescents previously under treatment for atopic asthma and now completely free of asthma related symptoms and medication for at least 12 months before enrolment) and healthy controls. The clinically cured group still has pathological signs highly compatible with asthma! (reproduced with permission-American Thoracic Society)*

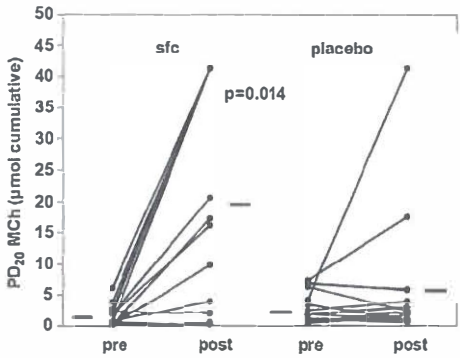


Figure 2.3 *Young adults with a history of atopic asthma, but without asthma related symptoms and medication for at least 12 months and documented bronchial hyperresponsiveness in challenge tests took part in the study. They were randomized to receive fluticasone-salmeterol treatment or placebo during three months. The active treatment reduced BHR in these ontherwise asymptomatic individuals!Each line respresents an individual, horizontal bars represent mean values (reproduced with permission-Elsevier).*

ICS and smoking

Active cigarette smoking has been associated with the development of asthma.[21,22] International guidelines of asthma management emphasise ICS as the most effective anti-inflammatory therapy for chronic asthma.[3-5] However, several studies have shown that the efficacy of corticosteroids is reduced in asthmatics who are active cigarette smokers *(Figure 2.4)*.[23-26] In an observational study in 75 asthmatic subjects, of whom 39 were smokers and 78 healthy controls of whom 30 were smokers, Livingston et al. [2007] demonstrated that smokers with asthma have an impaired cutaneous vasoconstrictor responses to topical steroids compared with never-smokers with asthma.[27] Besides this systemic effect of smoking, it was shown in this study that there is a reduced response to oral corticosteroids in smokers with asthma compared with never-smokers, measured by morning PEF, rescue medication use, daytime symptoms and asthma control score but not by FEV_1. In a review paper by Thomson et al. [2004] the negative influences of active smoking in asthmatics was discussed.[28] The authors stated that "Every effort should be made to encourage asthmatics who smoke to stop, although the effects of smoking cessation upon reversing the adverse effects of tobacco smoke on asthma control, therapeutic response to corticosteroids and airway pathology have yet to be fully elucidated. Alternative or additional therapies to inhaled corticosteroids are needed for asthmatic patients who are unable to quit smoking".[28] Cross sectional studies show that children and adults with asthma have on average lower lung function than non-asthmatics.[29,30] The annual decline of lung function in adults with persistent asthma is greater than in healthy adults, with an even faster decline in women than in men. Dijkstra et al. [2006] have shown in a 23 yr follow-up study that treatment with ICS in adult patients with moderate to severe asthma is associated with a reduction in the annual decline in FEV_1.[31] This was only found in adult men with < 5 pack years of smoking at follow-up. The effect was dose dependent and was not present in women or in men with ≥ 5 pack years of smoking. The decline in FEV_1 before start of ICS was not significantly different between men and women with < 5 pack years smoking. So, asthmatic women are not only more susceptible to cigarette smoke but the efficacy of corticosteroids seems to be less than in men. If adult asthmatic women who actively smoke are so much more vulnerable to tobacco smoke than men, and if smoking abolishes the anti-inflammatory effects of ICS, what would be the effects of passive smoking to asthmatic children, especially girls? It seems biologically plausible that even more negative influences on ICS effects in asthmatic girls who are exposed to cigarette smoke occur, although no firm data are available in the literature to date. The clinical emphasis should remain in motivating parents of asthmatic children to refrain from smoking both for themselves and their children.

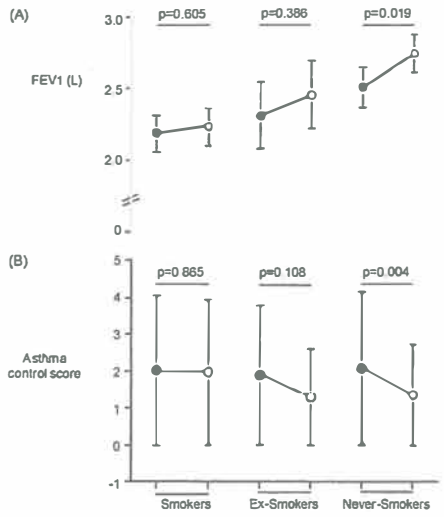


Figure 2.4. *This figure shows the change in FEV_1 (upper panel) and asthma control score (lower panel) for three groups of adults with asthma: smokers, ex-smokers and never-smokers. Active smoking impairs the efficacy of two week treatment with oral corticosteroids (reproduced with permission-American Thoracic Society).*

ICS and side effects

ICS proved to be effective and safe in children, both in the short term as well as the long term. In the past this view has sometimes led to the prescription of rather high doses of ICS. More recent guidelines advise lower doses of ICS to gain optimal asthma control. ICS side effects are dose-dependant, but individual sensitivity plays an important role. Although much experience with ICS treatment in children has been gained over the past decades, the clinician should always consider potential side effects. Well known, and relevant, in children are growth reduction, adrenal suppression, negative effects on bone metabolism, and local effects such as candidal infection of the throat and hoarseness of the voice.[7,32,33] Visser et al. [2004] showed that fluticasone at daily dosages of ≥500 mcg may have negative influence on bone metabolism and growth velocity.[7] Although temporary and reversible, this indicates that caution should be exercised when considering the prescription of higher dosages of ICS in children. However, under-treatment of asthma may also cause growth impairment. Reassuringly, long-term treatment with appropriate doses of ICS does not negatively influence final adult height.[3,4] Nonetheless, local side effects may occur even with low ICS dosages. Less well known side effects, that are sometimes mentioned, include hypertrichosis, skin bruising and behavioural problems including aggression and anxiety. However, in a large study of ICS side effects in the Netherlands, de Vries et al. [2008] could not find differences between asthmatic children on ICS and children reviewed in an otolaryngology outpatient clinic. Both groups the children had significantly more behavioural problems than in a healthy control group.[35]

LEUKOTRIENE RECEPTOR ANTAGONISTS (LTRA)

Leukotrienes are very potent mediators through interaction with the Cystinyl leukotriene receptor 1. Neither the synthesis nor the leukotriene induced actions are responsive to steroid treatment.[36] LTRA are the first new anti-inflammatory drugs since the introduction of ICS and the first to target a specific type of mediator. LTRA have both anti-inflammatory and bronchodilating properties. Leukotrienes act by binding to transmembrane spanning receptors, on multiple cell types, both inflammatory (neutrophils, basophils, and eosinophils), as well as structural cells (epithelium, endothelium and smooth muscle). They are registered for children from the age of six months to two years upwards, but will be available in a sprinkle form for the infants in the near future. LTRA are registered for the treatment of allergic asthma, exercise induced bronchoconstriction and allergic rhinitis, both as ad-on and (only in mild cases) as first line anti-inflammatory therapy. LTRA are less potent as an anti-inflammatory preparation than ICS, have less bronchodilating potential than salbutamol, but the combined effects for a once daily oral drug and the favourable side effect profile makes LTRA a potentially attractive choice as monotherapy in children with mild asthma and rhinitis. For young children, a useful feature of montelukast is that it is anti-inflammatory but lacks the potential side effects of steroids.[37] Bisgaard et al. [2005] have shown in a multicentre double-blind parallel group study that montelukast was effective in preschool children aged 2-5 years with a history of intermittent viral wheeze.[38] Children were randomised to receive either montelukast or placebo once daily for 12 months. During the study period, montelukast significantly reduced the asthma exacerbation rate, as well as the use of ICS and β_2-agonist. However, these favourable results were not applicable to children with symptoms of post- Respiratory Syncytial Virus bronchiolitis in children in a study by the same author.[39] In a randomised controlled trial in ninety-six 2-5 year-old children with asthma-like symptoms, Kooi et al. [2008] could not find significant differences between maintenance treatment with fluticasone, montelukast or placebo, which indicates that anti-inflammatory therapy in these children is not indicated while symptomatic treatment with salbutamol seems to be sufficient.[40] The group is probably too heterogeneous, but so are the children that present to clinicians with signs and symptoms in the out patient clinic. In future studies, subgroups of children who do benefit from anti-inflammatory treatment may be better defined. In a recent systematic review on montelukast as add-on therapy to ICS treatment in adults with mild to moderate asthma the authors concluded that montelukast added to ICS improved asthma control compared with ICS monotherapy[41], although the addition of a LABA to ICS is clinically at least as effective as the addition of montelukast. LTRA therapy may however have a better long term safety profile, which may be even more important in children than in adults.

MACROLIDES

Besides direct anti-microbial activity against gram-positive cocci and atypical pathogens, macrolides also have immune modifying effects. This has first been demonstrated with erythromycin in diffuse pan-bronchiolitis, and later in cystic fibrosis (CF). Macrolides bind to a subunit of bacterial ribosomes, ultimately leading to inhibition of bacterial protein synthesis and thereby direct antimicrobial activity.[42] The role of chronic infection with Chlamydia- and Mycoplama pneumoniae in the persistence or severity of asthma has been demonstrated.[43] The other proposed mechanisms in asthma besides this direct antimicrobial activity are effects on bronchial cells and on cells of the innate immune system.[44] Both in CF and in diffuse pan-bronchiolitis improvement has been reported with antibiotic levels below the minimal inhibitory concentrations of several bacteria, suggesting an anti-inflammatory activity as the putative mechanism of improvement. Not surprisingly, a Cochrane review found insufficient evidence to support or to refute the use of macrolides in patients with chronic asthma due to the small numbers

of patients' studied.[45] In the recent international guidelines on treatment of asthma a place for the role of macrolides has not been specified.[3-5]

CROMONES

The cromones, cromolyn sodium and nedocromil sodium, are mast cell stabilizers. Their effect is caused by phosphorylation of a cell membrane protein that is responsible for the termination of mediator release from mast cells, thereby turning off a very early stage of the asthmatic inflammatory response. Secondly, cromones are chloride channel blockers. When the opening of chloride channels with cell activation is prevented the subsequent calcium channel opening resulting in mast cell degranulation does not occur. However, their anti-inflammatory effect is weak[46] or nonexistent.[47] More importantly, publication bias is suggested by using a so called funnel plot showing an under representation of small studies with negative results. Therefore, beneficial effects of sodium cromoglycate as maintenance therapy in childhood asthma may have been overestimated.[48]

THEOPHYLLINE

It has been suggested that "The use of theophylline to treat asthma has undergone several cycles of enthusiasm and unpopularity over the past 50 years".[49] Currently, theophylline seems to be increasing in popularity again.[3-5] It is relatively cheap and is taken orally, but the major limitations of the drug are its narrow therapeutic index and wide inter-patient pharmacokinetic variability. As a result, dosing must be individually titrated to steady-state serumconcentrations to achieve both benefit and safety. In patients receiving theophylline monotherapy, sustained release doses providing a peak serum concentration of 10 to 20mg/L (mcg/mL) are best documented to improve symptoms and reduce the need for rescue therapy. Theophylline has bronchodilatory properties, though not as strong as long acting inhaled beta2 agonists. Antiinflammatory and immunomodulating effects have been described with serumlevels of 5mg/L. Therefore, serumconcentrations of 5 to 10 mg/L may be adequate for some patients, particularly if they are also receiving ICS. The molecular mechanisms for the anti-inflammatory action of theophylline are unclear, but the end result is suggested to be decreased inflammatory gene expression.[50]

OTHER ANTI-INFLAMMATORY DRUGS: BEYOND THE GUIDELINES

Metotrexate[51] and cyclosporine[52] have been used with a small but significant effect in the treatment of severe, persistent asthma in children. For others including chloroquine, azathioprine, dapsone, antiTNF-alfa, interferon-gamma and simvastatine there is a lack of studies to give guidance on the effects of use in children. Potential new cytokine therapeutic drugs and targets in asthma are reviewed elsewhere.[53]

CONCLUSIONS

ICS remain the most powerful anti-inflammatory drugs balanced against safety in asthma. Antileukotrienes may be an alternative when an oral drug is needed in case of mild asthma, especially when viral infections are the most important trigger. On a group level, neither ICS nor LTRA were different from placebo in 2-5 year old children: children appear to get better with time alone. Cromones seem to be of little value when studied on a group level. Theophylline is limited by its risk of side effects and the need for serum levels leaving it at best as a non-preferred alternative. Macrolides are of potential interest because of the combination of anti-infectious and anti-inflammatory properties in difficult to treat asthma but they can not be recommended as mainstream therapy at this point in time. It is worth noting that asthma cannot be prevented or cured by any of the currently available anti-inflammatory therapies. Nonetheless, when treatment is stopped, the disease manifestations return.

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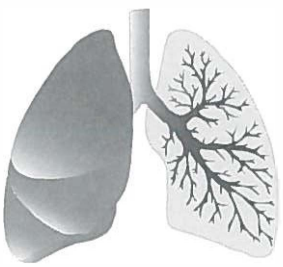
CHAPTER 3



Comparative in vitro evaluation of four corticosteroid metered dose inhalers: consistency of delivered dose and particle size distribution

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Abstract

Introduction

Recent developments concerning pressurized metered dose inhalers (pMDIs) with inhaled corticosteroids (ICS) are the introduction of ciclesonide and the replacement of propellants. As the results of in vivo studies depend on pMDI performance, it is necessary to evaluate pMDIs in vitro for delivered dose and particle size distributions

Methods

Fluticasone 125 µg, budesonide 200 µg, beclomethasone HFA100 µg, and ciclesonide 160 µg were compared for delivered dose and particle size using laser diffraction analysis with inspiratory flow rates of 10, 20 and 30 l/s.

Results

The volume median diameter of fluticasone was 3.5 µm, budesonide 2.8 µm, beclomethasone and ciclesonide both 1.9 µm. The mouthpiece retention was up to 30% of the nominal dose for beclomethasone and ciclesonide, 11–19% for the other pMDIs. Lifespan, flow rate, and air humidity had no significant influence on particle size distribution. The delivered dose of beclomethasone, budesonide, and ciclesonide remained constant over the lifespan. The delivered dose of fluticasone 125 decreased from 106% to 63%; fluticasone 250 also decreased whereas fluticasone 50 remained constant.

Conclusions

There is a significant difference in median particle size distribution between the different ICS pMDIs. Air humidity and inspiratory flow rate have no significant influence on particle size distribution. Ciclesonide 160 and beclomethasone 100 deliver the largest fine particle fractions of 1.1–3.1 µm. The changes in delivered dose during the lifespan for the fluticasone 125 and 250 may have implications for patient care.

INTRODUCTION

For the treatment of asthma the inhalation of medication is preferred and its effectiveness is widely appreciated.[1,2] The preferred size of the inhaled particles for deposition in both central and peripheral airways is claimed to be 1–5 μm for adults,[3] and 1.1–3 μm for children.[4,5] The delivered dose as percentage of the label claim as well as the particle size distribution (PSD) within the delivered dose has major influence on the site and amount of drug deposited in the airways.

Recent developments include the switch from chlorofluorocarbon (CFC) to hydrofluoroalkane (HFA) containing formulations for environmental reasons. This switch resulted in dramatic changes in plume properties[6] and particle size distributions in the aerosol.[7] For instance, for beclomethasone dipropionate (BDP) the same serum levels from a CFC-MDI as from only half the dose with an HFA-MDI after inhalation have been reported.[8] As the results of in vivo studies are largely dependent on pMDI performance, a change in pMDI should be thoroughly investigated to interpret in vivo studies with caution.

Furthermore ciclesonide (CIC), a new inhaled corticosteroid, was introduced. Ciclesonide is a prodrug which is converted into the active metabolite in the airways. Its high protein binding allows its use only once daily. It has been formulated as an aerosol solution for a pMDI with a hydrofluoroalkane as propellant, and most of the particles produced are claimed to be between 1 and 5 μm.[9]

To the best of our knowledge, no studies have been published in which the consistency of delivered dose and physical properties of the aerosol of ciclesonide are compared with other aerosols. Information about delivered dose and PSD is essential for both in vitro studies and clinical trials.

This prompted us to compare the ciclesonide 160 μg/dose pMDI with three other widely used pMDIs containing ICS: fluticasone 125 μg/dose, budesonide 200 μg/dose, and beclomethasone 100 μg/dose on their fine particle doses (1–5 μm) and the influence of lifespan of the pMDI, flow rate, and relative air humidity on the aerosol properties.

MATERIALS AND METHODS

Pressurized metered dose inhalators were obtained from the local hospital pharmacy. The pMDIs tested were: fluticasone dipropionate 125 μg/dose (FP, Flixotide®, GlaxoSmithKline); budesonide 200 μg/dose (BUD, Pulmicort®, AstraZeneca); ciclesonide 160 μg/dose (CIC, Alvesco®, Nycomed); and beclomethasone dipropionate 100 μg/dose (BDP, Qvar®, Teva). The CIC and BDP pMDIs are CFC-free and contain clear drug solutions; FP contains a CFC-free suspension whereas BUD contains a suspension in a mixture of CFC propellants. *Table 3.1* compares some properties of the pMDIs studied.

Name	Shape of mouthpiece	Dimensions mouthpiece (mm)	Distance of nozzle to mouthpiece end (mm)	Propellants/solvents	Suspensions solution
Fluticasone	Oval flattened sides	15x10	21	Tetrafluorethane	Suspension
Budesonide	Oval	18x13	27	Trichlorofluormethane Dichlorodifluormethane	Suspension
Beclomethasone	Circular	16 diameter	33	Norflurane / ethanol	Solution
Ciclesonide	Circular	16 diameter	33	Norflurane / ethanol	Solution

Table 3.1 *Some physical properties of the metered dose inhalers and actuators studied, measurements in millimetres.*

MEASUREMENT OF DELIVERD DOSE

pMDIs were connected to a filter system with Gelman glass filters A/E, P/N 61663; diameter 50 mm (Gelman Sciences, Ann Arbor, Michigan, USA). The filter system was supplied with a flow control unit and a solenoid valve with timer to set the flow rate to 20 l/min and suction time to 3 s. The suspension pMDIs were shaken for at least 10 s before doses were fired with the mouthpiece in a coupling flange with seal to prevent suction of false air and loss of aerosol. Time between firing of subsequent doses was at least 60 s to prevent excessive cooling of the pMDIs.

For three different devices (of the same batch) per type of inhaler, 15 individual doses were collected in the filter and analysed subsequently: five doses were taken from the beginning, five from the middle and five towards the end of the lifespan of the pMDI. The remaining doses in between were collected in groups of 5–40 and analysed together. The first ten doses of each new pMDI were wasted. Filter and mouthpiece retentions were dissolved in ethanol (analytical grade) and the drug solutions were analysed with a spectrophotometer (Unicam UV 500, ThermoSpectronic, UK) at 236 nm for fluticasone, 244 nm for budesonide, 243 nm for ciclesonide, and 239 nm for beclomethasone after it was checked with standard drug solutions added to filter depositions, so that the propellants in the drug formulations did not disturb the spectrophotometrical analysis. Checks have also been done on filter adsorption and the release of ethanol soluble components from the plastic mouthpiece parts.

PARTICLE SIZING IN THE AEROSOLS

For measurement of the particle size distributions (PSDs) in the aerosols from the pMDIs we used a laser diffraction apparatus (Sympatec Helos BF Magic) with inhaler adapter (Inhaler 2000, Sympatec, Clausthal-Zellerfeld, Germany). The inhaler adapter has a flow controller and solenoid valve with timer to adjust to the desired inspiratory flow rate and suction time. The flow rate was measured with a venturi-meter. Unless indicated otherwise, suction of the aerosol through the laser beam was at a constant flow rate of 10 l/min during 3 s. This was decided after it was checked with time sliced measurements that this time is sufficiently long enough to draw the entire aerosol from the pMDI through the laser beam. All measurements were started on an optical signal on detector channel 30 (for fine particles) of 0.2%. The

relative humidity in the laboratory (at a room temperature of 20–22 °C) was varied between low (approx. 30%), median (approx. 55%) and high (approx. 75%) to investigate the effect of ambient conditions on the particle size distribution.
A 100 mm (R3) lens was used with a measuring range of 0.5–175 µm and calculations were made with the Fraunhofer theory after it was checked that no overestimation of fine particles occurred. Ghost peaks from propellant(s) were removed with forced stability. No sheath or counter flow through the adapter was added to the aerosol cloud and the distance from the exit of the mouthpiece to the laser beam was fixed to 50 mm after it was checked that this yields realistic size distributions. The pMDI was fired manually and the particle size distributions in the aerosol were measured for five doses taken from the beginning, five from the middle and five towards the end of the lifespan of the pMDI. Additionally, the effect of flow rate (10, 20, and 30 l/s) on the PSD was determined. Data per flow rate are the mean of 3 series of 5–10 doses in which each series is for a different pMDIs from the same lot. PSDs are presented as X_{10}-, X_{50}- and X_{90}-values derived from the cumulative volume distribution curves as a function of the particle diameter.

Statistics were performed using SSPS for Windows (version 13.0) and all tests were performed two sided, a $p < 0.05$ was considered statistically significant.

RESULTS

Figure 3.1 shows the mean metered dose (with maximum and minimum values obtained) as percent of the label claim from the three different devices of the same batch for all four pMDIs. In *Figure. 3.1* the mean, maximum and minimum per device are for all doses, except for the first ten which were intentionally wasted. As expected there is a spread in metered dose for every individual pMDI. The variation is particularly extreme for at least two of the FP pMDIs, varying from 62% to 158%, respectively from 72% to 162% of the label claim for the devices 1 and 2, this is statistically significant. The mean metered dose is approximately 10% higher for CIC than for the other pMDIs, but the mouthpiece retention in this inhaler is higher too: 30% versus 11–19% for the other pMDIs. As a result, the delivered dose from CIC is more or less the same as that from the FP and BUD pMDI: on average 85% of the label claim. Only the BDP pMDI has a slightly lower delivered dose of 70% of the label claim.

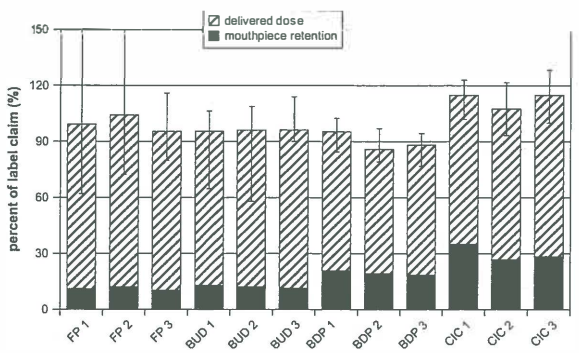

Figure 3.1 *Mean metered dose (for all doses) as percent of label claim for three different devices of the same type taken from the same lot with spread bars indicating the maximum and minimum values obtained.*

FP = fluticasone 125 µg/dose;
BUD = budesonide 200 µg/dose;
BDP = beclomethasone 100 µg/dose;
CIC = ciclesonide 160 µg/dose.

Figure 3.2 a–d shows the consistency of the delivered doses for all devices within their lifespan. It can be seen that there is a small, not clinically significant spread between the delivered doses of BDP and CIC and that two BUD pMDIs exhibited a low output at the start after having already wasted the first 10 doses, one of them was statistically significant. The delivered dose of FP pMDI decreased statistically significantly

with the number of doses taken to less than 70% of the output in the first actuations. Because we were surprised by this result we studied twelve additional FP 125 µg pMDIs taken from different lots, and also measured (in duplo) the delivered dose of FP 50 µg and 250 µg for comparison.

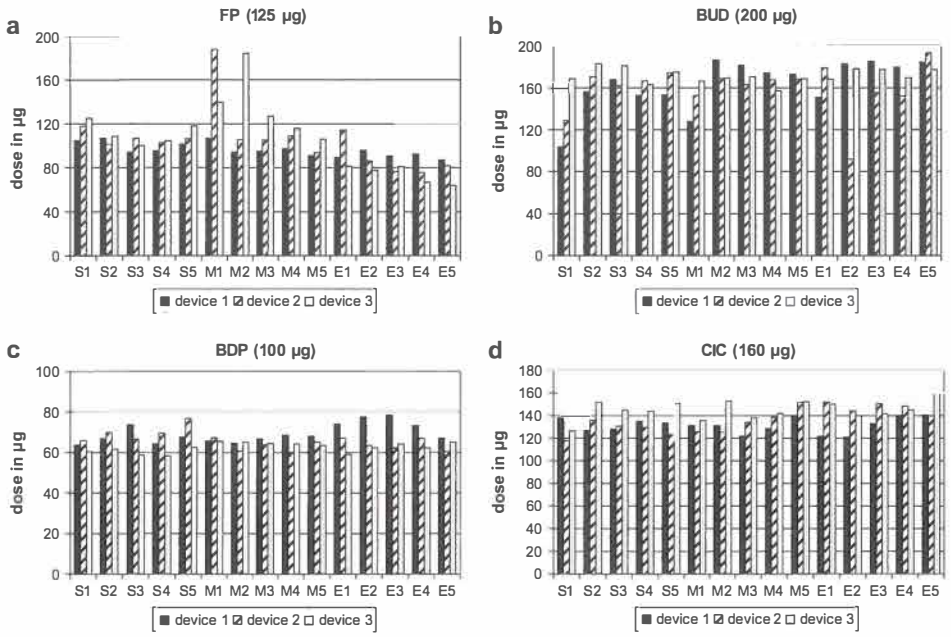

Figure3.2 *a–d: Delivered dose in µg at the start (S1–S5), the middle (M1–M5) and the end (E1–E5) of the lifespan of the same device. FP = fluticasone 125 µg/dose; BUD = budesonide 200 µg/dose; BDP = beclomethasone 100 µg/dose; CIC = ciclesonide 160 µg/dose.*

Figure 3.3 summarises the mean values with spread bars showing maximum and minimum doses obtained. It was found that the output of FP 50 remains constant. However, the delivered dose of FP 250 also decreased statistically significant with the number of doses taken from the device.

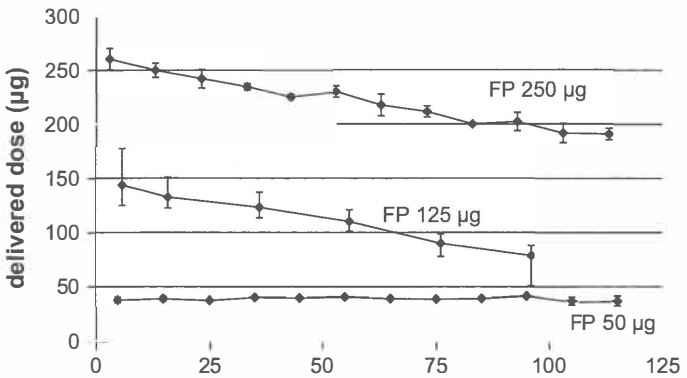

Figure 3.3 *Changes in the delivered dose from fluticasone (FP) 50, 125 and 250 µg with the cumulative number of doses taken from the same device. Data points for FP 125 µg are the mean of 15 devices (from five different batches); those for FP 50 and 250 µg are the mean of two devices (from different batches). Spread bars indicate the maximum and minimum values obtained.*

Characteristic values (X_{10}, X_{50} and X_{90}) from the cumulative volume distributions in the aerosol are presented in *Figure 3.4* The X_{50} of FP (3.5 µm) is significantly greater than all others, and the X_{50} of BUD (2.8 µm) is significantly greater than those of BDP and CIC (1.9 µm for both devices). Also the spread in droplet size is much smaller for BDP and CIC. FP 125 µg showed a great inter-device spread in size distribution; mean values for the median droplet diameter varied between 2.1 and 3.8 µm for individual devices from different batches, this is statistically significant. For BDP and CIC the inter-device variation with respect to PSD was negligible, whereas the X_{50}-value for BUD ranged from 2.7 to 3.0 µm which is statistically significant. The data in *figure 3.4* are for one particular device producing a representative aerosol and each data point in *figure 3.4* is the mean of five doses taken from the start (S), middle (M) or end (E) of the inhaler's lifespan. We also checked the size distributions in the aerosols from the FP 50 and 250 µg pMDIs and found that the FP 50 µg has a much smaller median diameter (X_{50} is 2.7 µm) than the FP 125 µg. In contrast, the FP 250 appeared to deliver significantly larger particles with a median diameter of 4.2 µm (data not shown).

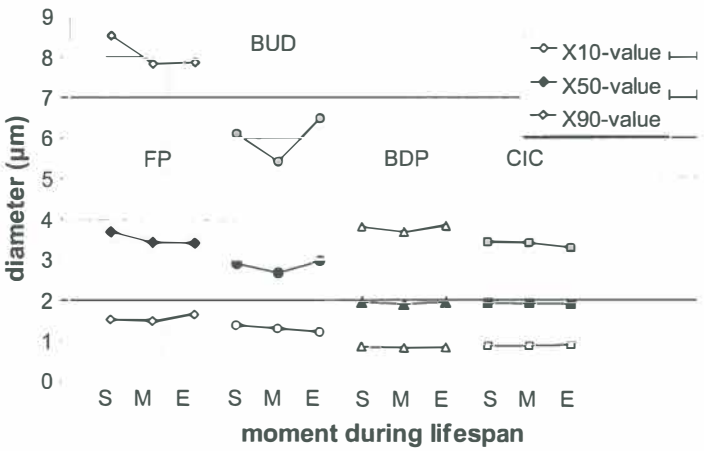

Figure 3.4 *Particle size distribution of within lifespan of the pMDI for fluticasone (FP) 125 µg, budesonide (BUD) 200 µg, HFA beclomethasone (BDP) 100 µg, and ciclesonide (CIC) 160 µg at a suction of 10 l/ min through the laser beam. S, M and E refer to doses taken at the start, middle and end of the lifespan respectively.*

Figure 3.4 also shows that the PSDs of all four pMDIs remained rather constant within their lifespan. The relative air humidity within the range from 30 to 75% had no significant influence on particle size distribution. For FP and BUD the inter-device variations were significantly larger than for BDP and CIC.

The influence of the flow rate on the particle size distribution in the aerosol is shown in *Figure 3.5*. This figure demonstrates the small effects of the flow rate on the PSD for all 4 tested devices within the range between 10 and 30 l/min. Due to differences in metered dose, mouthpiece retention and size distribution in the aerosol, different fine particle mass fractions (FPF < 5 µm as percent of label claim) may be expected. This is shown in *Figure 3.6*.

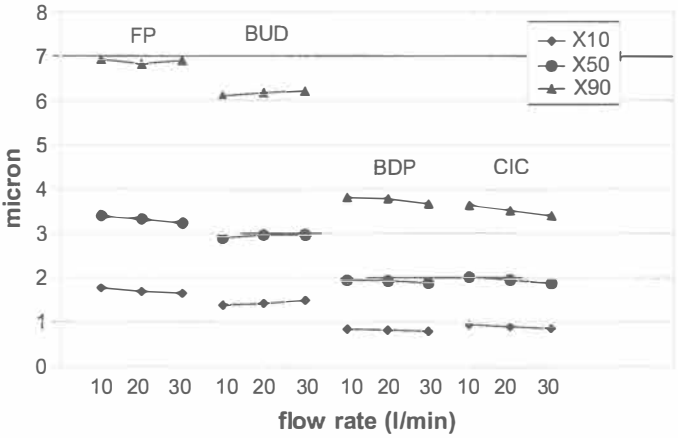

Figure 3.5 *The particle size distribution in the aerosol as function of the flow rate. FP = fluticasone 125 µg/dose; BUD = budesonide 200 µg/dose; BDP = beclomethasone 100 µg/dose; CIC = ciclesonide 160 µg/dose.*

All three pMDIs of the same type showed a very high consistency in mean delivered fine particle mass fraction, the fraction < 5.0 µm being highest for the CIC pMDI. The aerosols from the FP and BUD pMDI contained only very few particles <1 µm but for the BDP and CIC pMDI nearly 15% of the delivered aerosol was in the particle range of <1 µm. There were also considerable differences for the fraction 3.1–5 µm, between FP and BUD on the one hand (respectively 29% and 24% of the label claim) and BDP and CIC on the other (approx. 10% for both). For the fraction 1.1–3.1 µm the CIC pMDI scored best (55%), followed by BDP (44%), BUD (42%) and FP (39%).

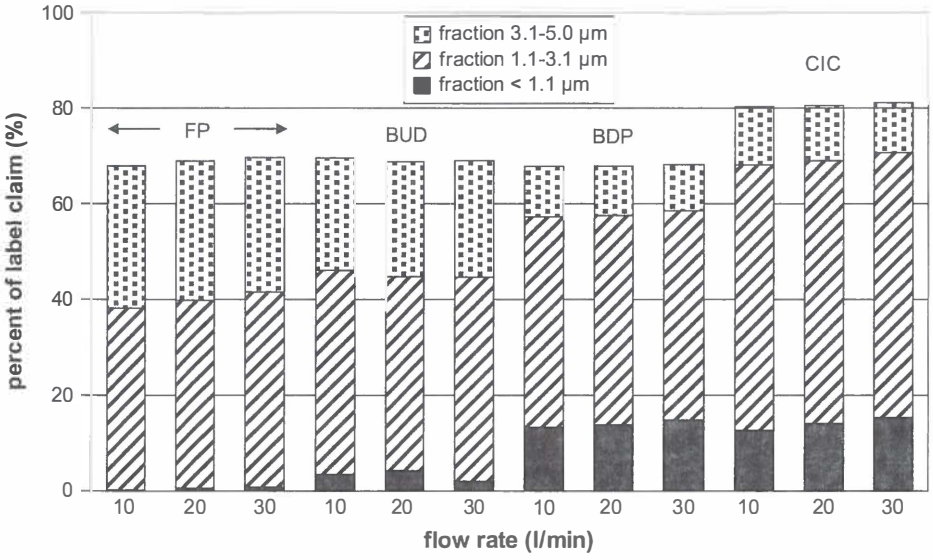

Figure 3.6 *The size distribution within the fine particle fraction as percent of label claim based upon the mean delivered dose from the pMDI at 20 l/min. FP = fluticasone 125 µg/dose; BUD = budesonide 200 µg/dose; BDP = beclomethasone 100 µg/dose; CIC = ciclesonide 160 µg/dose.*

DISCUSSION

To our knowledge, this is the first study to compare the in vitro performance of four different ICS pMDIs including FP, BUD, BDP, and CIC at three different flow rates and taking lifespan and ambient humidity conditions into account. Most previous studies dealt with single pMDIs. [10-15] Terzano studied the particle characteristics of FP, BDP, and flunisolide in vitro at two different flow rates (30 and 60 l/min).[16] Feddah compared the in vitro performance of three ICS DPIs with pMDIs containing the same drugs (FP, BUD and BDP) in an impactor at three different flow rates (30, 60 and 90 l/min) but their study did not include BDP and CIC.[17] Dalby, Barry and Stein also compared the performance of three different pMDIs, but their comparisons included other types of drugs, like salmeterol, salbutamol, and cromolyn sodium.[18-20]

Figure 3.1 shows that none of the pMDIs tested delivered a mean dose corresponding with the label claim. The spread found in delivered dose was relatively high for FP and also significant for BUD. For the BUD pMDI this was the result of a random variation, showing a few extremes towards lower values varying between 52% and 79% of the label claim *(Figure 3.2b)*. However, for FP a gradual and significant decrease in delivered dose was obtained over the lifespan of the pMDI. This decrease appeared to be consistent for 15 devices taken from five different batches as shown in *Figure 3.3*. On average (for all fifteen devices) the mean of the doses 11–20 from this pMDI was 106% of the label claim versus only 63% for the mean of the doses 92–100. This reduction is not only statistically significant, but also of clinical importance. We did not find an explanation for the inconsistencies found. However, these results point at the importance of knowing which doses from FP and BUD are used for in vitro studies as well as for clinical trials.

For all four types of pMDIs the delivered dose is lower than the metered dose due to some retention in the mouthpiece. As summarised in *Table 1* mouthpieces have different shapes and dimensions; only those of CIC and BDP are the same. We found a 15% reduction for the mouthpiece retention of BDP and CIC by shortening the distance of the nozzle to the mouthpiece end to 21.5 mm. Shortening also appeared to influence the PSD in the aerosol. For BDP the X_{50}-value decreased from 1.95 to 1.79 µm; for CIC the decrease was from 2.02 to 1.8 µm. Mouthpiece shortening may also have consequences for the deposition in the mouth and valved holding chambers however. The net result of all these effects has to be studied.

Regarding the particle size distributions in the aerosols *(Figure 4, Figure 5* and *Figure 6)* previous findings obtained with cascade impactor analysis are confirmed.[10,17,19,20] BDP and CIC pMDIs deliver much finer particles than FP and BUD pMDIs. The data in *Figure 6* suggest that BDP and CIC produce the highest mass fractions within the size rang of <3.1 µm and therefore are most appropriate for use in children because of their smaller airways. The relatively high fraction of particles <1.1 µm in the aerosols from the BDP and CIC is likely to be exhaled to a large extent again, unless a relatively long breathhold period is established. [21,22]

The PSDs for all four pMDIs do change statistically significant, but we do not think this is clinically relevant within their lifespan, nor when the flow rate or relative humidity is varied between 10 and 30 l/min, respectively 30 and 75%.

In contrast with most previous studies we used a laser diffraction technique for the characterisation of the particle size distribution in the aerosol. With the laser diffraction technique the size distribution of individual doses can be measured more reproducibly than with impactors, which suffer from variable

losses in the valve, actuator, and USP throat.[20] Therefore, the laser diffraction technique enables a better assessment of the spread in the fine particle dose in the emitted aerosol. There is a discrepancy between previously published data from cascade impactor analysis and the laser diffraction data in this study for the same pMDIs. For instance, we found a volume median diameter of 1.9 µm for BDP *(Figure 4)*. Leach and Stein referred for BDP to an average particle size of approximately 1 µm; and from their cascade impactor data mass median aerodynamic diameters (MMADs) of 0.9–1.0 µm can be calculated.[7,20] The reason for the different results may be losses in the induction port. For BDP these losses are within the range from 20 to 30% of the label claim (at approx. 30 l/min). Partly as a result of that, cumulative fine particle fractions collected in the impactor were only between 40 and 50% of the label claim.[20] With the laser diffraction technique there are no losses in an induction port and the mean delivered dose for BDP in our study was more than 70%. Possibly classification occurs in the throat of an impactor, as a result of which the size distribution within the remaining aerosol fraction is changed. Moreover, the evaporation during longer travelling distance in cascade impactors is likely to result in an overestimation of the fine particles. There are more basic differences between cascade impactor and laser diffraction technique, but it is very unlikely that the differences in median diameters obtained with both techniques are the result of that.

CONCLUSIONS

In this study we show that the in vitro evaluation of ICS pMDIs under well-controlled conditions is mandatory for adequate evaluation of drug delivery studies in patients. We have demonstrated that the performance may vary over the lifespan of the pMDI and the fine particle fraction differs relevantly between different ICS. Design, such as the length of the mouthpiece, significantly influences dose delivery. We found that the delivered dose of FP 125 and 250 decreased significantly during its lifespan, and in some of the BUD pMDIs the first delivered doses were significantly lower. Although our data are consistent, they have to be confirmed by others and the clinical relevance should be investigated. The particles of CIC and BDP are the most appropriate for paediatric use. Our results partly confirmed earlier studies.

Before testing a combination of valved holding chambers and pMDIs, the performance of the pMDI itself should be known in relation to the environmental conditions and the inspiratory flow rate. Furthermore it is important to realise that there may be a significant inter-dose spread as well as inter-device variations regarding delivered dose and PSD. Particularly an inter-device spread makes it necessary that the same pMDI is used for comparison of data obtained with and without holding chamber. Knowledge of in vitro performance is essential to value important in vivo studies.

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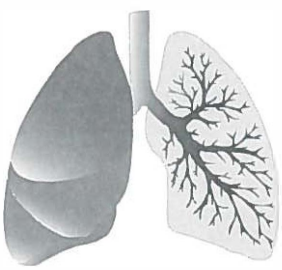
CHAPTER 4



High air humidity increases delivered total and fine particle dose of inhaled corticosteroids from valved holding chambers

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Abstract

Background

Optimal clinical effects of inhaled corticosteroids (ICS) in patients with asthma depend on their deposition in both large and small airways. This requires a high fine particle dose in the aerodynamic size range 1-3µm. We studied the effect of relative air humidity, lag time and flow rate on the delivered fine particle dose from 6 ICS pressurized metered dose inhalers (pMDI)-valved holding chamber (VHC) combinations.

Methods

Fluticasone 125 µg, budesonide 200 µg, beclomethasone dipropionate 100 µg and ciclesonide 160 µg were delivered by pMDIs without VHCs and with fully primed VHCs. Aerosol doses were collected on filters at low (25-35%), medium (55-65%) and high (74-80%) relative air humidity and analysed using UV absorption. Particle size distributions of the aerosols were measured using laser diffraction with a lag time of 0 to 20s after firing the pMDIs. Suction flow rates were 10, 20 and 30 L/min.

Results

Increasing relative air humidity from 30% to 80% increased total delivered dose from VHCs by a factor 1.4 (mean value of all combinations; $p<0.002$; range 1.0-2.0) without significantly changing the particle size distributions. Fine particle doses from VHCs at medium relative humidity were 16.9% of label claim from pMDI for fluticasone (Volumatic®), 18.6% for budesonide (Nebuhaler®), 24.5% for beclomethasone (AeroChamber®), 36.6% for ciclesonide (AeroChamber®), all comparisons $p<0.001$. Suction flow rate did not change the Volume Median Diameter (VMD) of ICS from VHCs. The VMD reduction after 20 seconds lag time was less than 5% (mean value).

Conclusions

High air humidity leads to an increased delivered total and fine particle dose (1-3 µm) from VHCs compared to low humidity. The subsequently higher ICS deposition in large and small airways may lead to better asthma control. We recommend using ICS from VHCs in humid environments, like in a bathroom.

INTRODUCTION

Asthma is an inflammatory disease affecting both large and small airways.[1,2] Targeting the small airways with inhaled corticosteroids (ICS) is regarded the important challenge of ICS treatment,[3-7] in particular as the number of ICS receptors increases towards the lung periphery.[8] Clinical studies suggest that ICS with a large fine particle fraction (1-3 µm) might be more effective than ICS with larger particles.[9-12] This is compatible with the observation that 1.5 µm ICS particles provide higher peripheral and higher total lung deposition than particles of >3 µm.[13]

Adequate inhalation technique is required for optimal inhaled drug treatment. Valved holding chambers (VHCs) are used to overcome frequently encountered coordination problems with the use of a pressurized metered dose inhaler (pMDI). Additional advantages of VHCs are that they allow for the retention of large particles in the VHC[14] and reduce the speed of the aerosol particles in the mouth and throat region[15], resulting in lower oropharyngeal deposition.

A disadvantage of VHCs is that they may reduce the total dose available for inhalation due to drug deposition onto the inner wall of the VHC caused by electrostatic charge deposition, sedimentation by gravity, and inertial deposition due to the high plume velocity.[16-18] This reduction of total dose is less when the VHC is washed or primed.[19,20] Relative humidity can affect electrostatic charge, but to what extent is yet unknown. Further, a lower inhalation flow rate and longer lag time between dose firing and inhalation from the VHC may decrease the fine particle dose from VHCs. These three factors, i.e. relative air humidity, inhalation flow rate and lag time, may thus reduce clinical efficacy of ICS when administered with a pMDI-VHC combination. Therefore, we studied these three factors on both total dose and fine particle dose delivered from six pMDI-VHC combinations and compared delivered dose from the VHC to label claim and delivered dose directly from the pMDI.

Our ultimate goal was to find the best combination and the best circumstances for optimal (fine particle) output, which may contribute to improved treatment of patients with asthma.

MATERIALS AND METHODES

pMDIs and VHCs

The hospital pharmacy provided pMDIs; the manufacturers provided the VHCs studied. The ICS tested were fluticasone dipropionate (FP) 125 µg/dose:120 doses (Flixotide®, GlaxoSmithKline); budesonide (BUD) 200 µg/dose: 200 doses (Pulmicort®, AstraZeneca); ciclesonide (CIC)160 µg/dose: 200 doses (Alvesco®, Nycomed); beclomethasone dipropionate hydrofluoralkane (HFA; BDP) 100 µg/dose: 60 doses (QVAR®, Teva). The beclomethasone and ciclesonide pMDI's have drug solutions in HFA with ethanol as co-solvent and yield fine particle aerosols: (volume median diameter for both drugs is 1.9 µm as measured with laser diffraction technique.[21] The fluticasone pMDI with an ethanol-free HFA suspension and the budesonide pMDI with a suspension in a mixture of CFC propellants, yield larger particle aerosols (volume median diameter 3.5 µm en 3.0 µm respectively.[21] We included fine and larger ICS aerosols in our study to investigate the influence of the particle size distribution on the output from VHCs. The suspension pMDIs were shaken for at least 10 seconds before doses were fired. Time between firing of subsequent doses was at least 60 seconds to prevent excessive cooling of the pMDIs due to propellant evaporation. The first ten doses of each new pMDI were wasted. The total number of doses used for each part of the study is summarised in the data collection protocol *(Table 4.1)*.

VHCs used were Volumatic® (Allen & Hanburys Ltd) with fluticasone, Nebuhaler® (AstraZeneca) with budesonide and AeroChamber Plus® (Trudell Medical International) with beclomethasone, ciclesonide, fluticasone and budesonide. The VHCs were either used new (unwashed, non-primed), fully primed (> 20 doses) or washed by dipping in a fresh, mild solution of liquid dish detergent in lukewarm clean water followed by drying to air. After the study was completed, the anti-static AeroChamber Plus Flow-Vu® became available. This VHC was tested unprimed and unwashed as instructed in the patient information leaflet.

Combination:	FP+VOL	FP+AC	BUD+NEB	BUD+AC	BDP+AC	CIC+AC
1. Effect of relative air humidity on delivered dose from VHC Flow rate: 20 L/min; suction time: 12 s; relative air humidity: 25-35%; 55-65% and 75-80%; lag time: 3 s						
Number of doses*	498	486	603	648	1026	468
2. Effect of lag time on delivered dose from VHC: Flow rate: 20 L/min; suction time: 12 s; relative air humidity: 50%; lag times: 1; 3; 5; 10 and 20 s.						
Number of doses*	105	105	100		105	110
3. Effect of washing and priming on delivered dose from VHC Flow rate: 20 L/min; suction time: 12 s; relative air humidity: 30%; lag time: 3 s.						
Number of doses*		90			90	90
4. Effect of flow rate on particle size distribution from pMDIs and VHCs Flow rates: 10; 20 and 30 L/min; suction times: 3 s (pMDIs) and 5 s (pMDI+VHC); lag time: 3 s						
Number of doses*	pMDI: 60 pMDI+VHC: 45	pMDI: 60 pMDI+VHC: 45	pMDI: 60 pMDI+VHC: 45	pMDI: 60 pMDI+VHC: 45	pMDI: 60 pMDI+VHC: 45	pMDI: 60 pMDI+VHC: 45
5. Effect of lag time on particle size distribution from VHC Flow rate: 10 L/min; suction time: 5 s; relative air humidity: 50%; lag times: 1; 3; 5; 10 and 20 s						
Number of doses*	60	60	60	60	60	60

*The total number of doses used depends on the number of doses in the pMDI; the first and last ten doses of each pMDI were excluded. The total number of doses from pMDIs used in this study: 5514

Table 4.1 *Data collection protocol.*

MEASUREMENT OF DELIVERED DOSES

pMDIs and VHCs were connected to a filter system with glass filters (Gelman Sciences, Ann Arbor, Michigan, USA). The filter system was supplied with a flow control unit and a solenoid valve with timer to set the desired flow rate and suction time. Suction time was adjusted to assure complete emptying of the total system *(Table 4.1)*. Single breath inhalation from a VHC is currently advised in clinical practice whenever possible instead or tidal breathing.[22]

Drug deposits on filter were analysed with a spectrophotometer (Unicam UV 500, ThermoSpectronic, UK) after dissolution in ethanol or water. The procedure was checked with standard drug solutions added to filter depositions, to make sure that the propellants in the drug formulations did not disturb the spectrophotometrical analysis. Checks and, if necessary, corrections were also done on filter adsorption and the release of ethanol soluble components from the plastic mouthpiece parts. We alternatingly measured 9 subsequent doses together from the pMDI and 9 together from the pMDI with VHC because of the previously reported large variation in delivered dose from pMDIs.[21] Each 10th dose was used to analyse the mouthpiece retention from the pMDI. We did so for all doses from the pMDI with two different devices from the same batch and averaged all data of two duplicate pMDI-VHC combinations. We also assessed delivered dose from unwashed, unprimed VHCs and compared them with washed VHCs.

MEASUREMENT OF PARTICLE SIZE DISTRIBUTION

Particle size distributions in the aerosols from the pMDIs and pMDI-VHCs, were measured with laser diffraction technique (HELOS BF MAGIC) using an inhalation adapter (INHALER 2000, both Sympatec, Clausthal-Zellerfeld, Germany). The inhalation adapter used has a flow controller and solenoid valve with timer to adjust the desired inspiratory flow rate and suction time. A 100mm (R3) lens was used with a measuring range of 0.5 to 175 mm. All measurements were started on an optical signal on detector channel 30 (for fine particles) of 0.2%. Calculations were made with the Fraunhofer theory after checking that no overestimation of fine particles had occurred. Ghost peaks from propellants were removed with forced stability. Further details are described in our previous study.[21] With laser diffraction technique Volume Median Diameters (VMD or X_{50}) are derived from the cumulative volume distribution curves and X_{10} as well as X_{90} define the size range of the aerosol. We did not aim to measure the absolute aerodynamic size distributions in the aerosols. Our aims were focussed on measuring the changes in the size distribution as function of the relative air humidity, flow rate and lag time, with and without VHC. Laser diffraction was used as these aims cannot be met in the same way and for the large number of doses with cascade impactor analysis.

All laser diffraction measurements were performed at medium relative humidity since humidity had a negligible effect on particle size distributions. The fine particle fractions were defined as the volume fractions between 1.0-3.0 μm.

To study the effect of the inspiratory flow rate on the volume median diameter, suction of the aerosol through the laser beam was set at a constant flow rate of 10, 20 or 30 L/min during 3 seconds for pMDIs and 5 seconds for VHCs. Time-sliced measurements showed that 5 seconds at 10 L/min are sufficient to obtain a representative measurement of the particle size distribution in the aerosol.

The distance from the exit of the pMDI or VHC mouthpiece to the laser beam was fixed at 50 mm. A longer distance did not change the particle size distribution, which suggests that droplet evaporation or particle growth did not occur.

DELIVERED TOTAL DOSE FROM VHC AND RELATIVE AIR HUMIDITY

Measurement of the effect of relative air humidity on delivered doses from (fully primed) VHCs was performed at a flow rate of 20 L/min. Humidity values were 25-35% (low), 55-65% (medium) and 75-80 % (high) in a room with temperature varying between 18.5 and 20.5 °C. All doses from two different pMDIs from the same batch were used for each pMDI-VHC combination at each humidity-condition and the data per combination (and condition) were averaged. After the study was completed, the anti-static AeroChamber Plus Flow-Vu® became available. The delivered dose from this antistatic device was compared with that from the previously used AeroChamber® for CIC and BDP at low (25%) and medium (65%) RH. CIC and BDP were used anticipating that the effect of electrostatic charge is largest for the smallest particles.

EFFECT OF LAG TIME AND VHC PREPARATION ON DELIVERED DOSE VHC

Total delivered dose from the fully primed VHCs as function of the lag time between firing of a dose and suction (1; 3; 5; 10 and 20 s) was measured at a flow rate of 20 L/min and at medium humidity. We also assessed the total delivered dose from unwashed and unprimed VHCs and compared them with washed VHCs

EFFECT OF FLOW RATE ON PARTICLE SIZE DISTRIBUTION

The effect of the inspiratory (suction) flow rate on the volume median diameter in the aerosols from pMDIs and pMDI-VHC combinations, was studied with constant flow rates of 10, 20 or 30 L/min at medium humidity.

EFFECT OF LAG TIME ON PARTICLE SIZE DISTRIBUTION

To study the effect of the lag time (1; 3; 5; 10 and 20 s) between firing of a dose and suction on the particle size distribution of the aerosol from the VHC at medium humidity, the inspiratory flow rate was set at 10 L/min.

FINE PARTICLE DOSE

The fine particle dose was calculated on the basis of measured delivered dose on filter and the fine particle fraction measured with laser diffraction analysis

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS, version 14. We used the Kruskall-Wallis test for comparison of more than two groups. Differences between 2 groups were analysed by means of the Mann-Whitney U test. A p-value of < 0.05 was considered significant.

RESULTS

The impact of relative humidity on delivered total dose from pMDI-VHC combinations

VHC output, expressed as percentage of pMDI output, increased with higher relative air humidity, except for budesonide with AeroChamber®. The overall mean VHC output, expressed as percentage of the delivered dose from the pMDI without a VHC, increased from a mean of 34% in all combinations at low humidity to a mean of 40.8% at medium and 47.6% at high humidity. These differences are highly

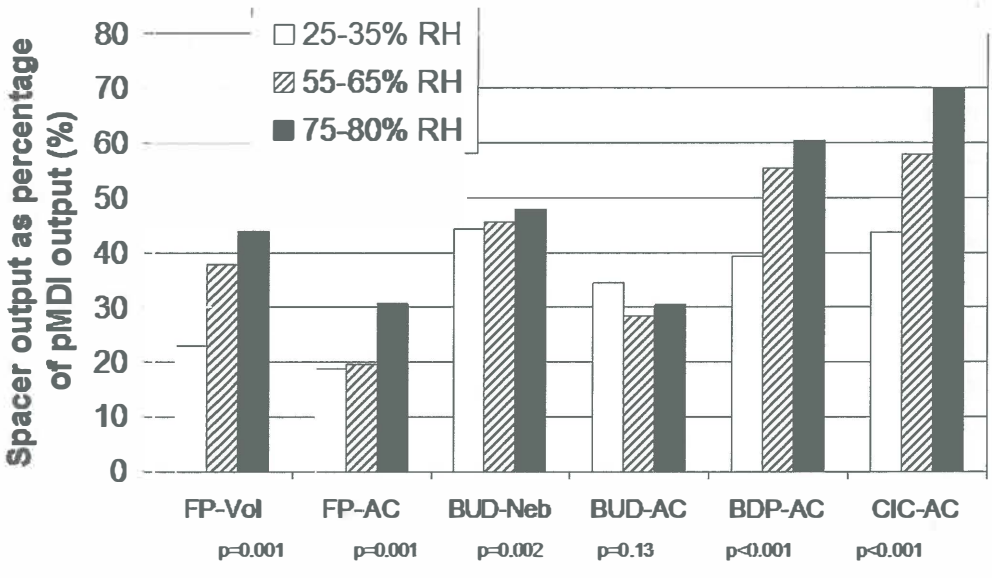

Figure 4.1 *Delivered doses from primed and washed VHCs as a percentage of the delivered doses from the pMDIs, for three different relative humidity (RH) ranges. Each bar is the mean of two devices from the same batch over the total lifespan (i.e. 200 doses per device for HFA-BDP and BUD; 120 for FP and 60 for CIC). The Kruskall-Wallis test was used for comparison of the 3 tested humidity ranges.*

AC =AeroChamber®,Plus; BDP = beclomethasone; BUD = budesonide; CIC = ciclesonide; FP = fluticasone; NEB= Nebuhaler®; pMDI = pressurised metered dose inhaler; RH = relative humidity of the air; VOL=Volumatic®

significant for all combinations, except for budesonide with the AeroChamber® *(Figure 4.1* and *Table 4.2)*. VHC output for fluticasone + Volumatic® increased with 102% from low to high relative humidity ($p = 0.001$), for fluticasone + AeroChamber® with 64% ($p = 0.001$) , for ciclesonide + AeroChamber® with 57% ($p < 0.001$), for beclomethasone + AeroChamber® with 54% ($p < 0.001$) whereas the change for budesonide + Nebuhaler® was lowest with 10% ($p = 0.002$).
BDP and CIC in the antistatic Aerochamber® Plus Flow Vu™ also showed an increase in delivered dose with increasing humidity: CIC output increased from 44% to 71% and BPD from 14% to 61% from low to medium humidity.

Drug-VHC	Humidity	VHC output % from pMDI mean (CI)	p-value between relative humidity ranges
FP-VOL	All		**p = 0.001**
	Low	22.9 (19.0-26.8)	low-medium 0.005
	Medium	38.0 (28.5-47.5)	low-high <0.001
	High	46.2 (40.9-51.5)	medium-high >0.1
FP-AC	All		**p = 0.001**
	Low	18.7 (12.2-21.2)	low-medium >0.1
	Medium	19.5 (15.1-24.0)	low-high 0.001
	High	30.7 (26.8-34.6)	medium-high 0.003
Bud-NEB	All		**p = 0.002**
	Low	44.2 (42.6-45.8)	low-medium >0.1
	Medium	45.7 (44.4-47.1)	low-high 0.001
	High	48.8 (47.2-50.4)	medium-high 0.019
Bud-AC	All		**p = 0.13**
	Low	34.6 (29.3-40.2)	
	Medium	28.4 (26.5-30.3)	
	High	30.2 (28.4-32.0)	
BDP-AC	All		**p < 0.001**
	Low	39.4 (37.3-41.5)	low-medium <0.001
	Medium	55.5 (52.7-58.3)	low-high <0.001
	High	60.5 (58.2-62.8)	medium-high 0.018
CIC-AC	All		**p < 0.001**
	Low	44.3 (42.7-45.9)	low-medium 0.001
	Medium	58.0 (54.4-61.6)	low-high 0.001
	High	69.5 (64.1-74.9)	medium-high 0.021

Table 4.2 *The impact of relative humidity on the pMDI+VHC output compared to the output from pMDIs without VHC. CI = 95% confidence intervals.*

DELIVERED FINE PARTICLE DOSE FROM pMDIs AND pMDI-VHC COMBINATIONS

Aerosols from pMDI-VHC combinations had (on average) a slightly smaller volume median diameter (VMD) than the aerosols directly from the pMDIs, except for the ciclesonide – AeroChamber® combination *(Figure 4.2)*. In addition, the fraction of particles < 1.1 µm increased for all pMDIs when delivered from the VHC *(Table 4.3)*. However, this hardly affected the volume percentages between 1.0 and 3.0 µm which are most relevant for peripheral lung deposition as well as the most homogeneous drug deposition over the whole lung.[13] Only fluticasone with Volumatic® showed an increase in fine particle dose (FPD) from 42.9% (for pMDI) to 49.9% for pMDI plus primed VHC ($p<0.01$) *(Table 4.3)*. The differences in delivered doses and in particle size distributions between the pMDIs alone and the pMDI-VHC combinations resulted in different delivered fine particle doses *(Table 4.3)*. The fine particle dose at medium humidity, using a fully primed or washed VHC, ranged between 44% (fluticasone-Volumatic®) and 60% (ciclesonide-AeroChamber®) of the fine particle dose directly from the pMDI.

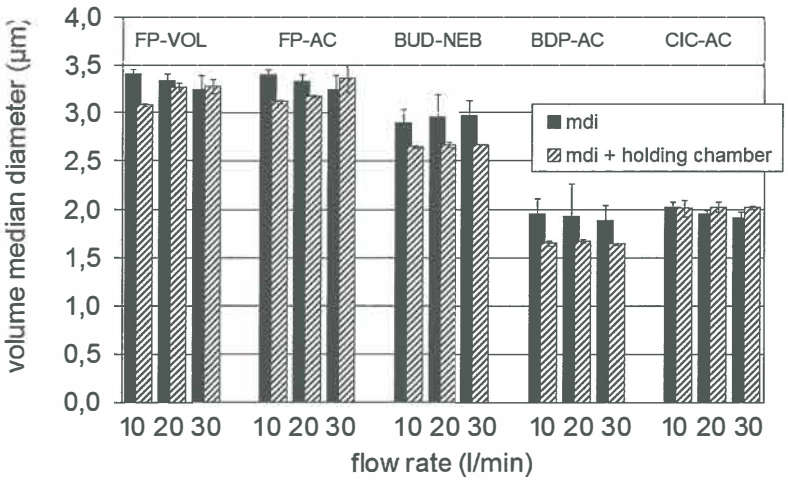

Figure 4.2 *Volume median diameters in the aerosols from fully primed VHCs (shaded bars) at three different flow rates compared to the volume median diameters in the aerosols directly from the pMDIs (black bars). All bars are the mean of three series of ten subsequent doses taken from two different pMDIs (totalling 60 laser diffraction measurements per bar). Five seconds suction time at a medium relative humidity.*

pMDI		Fluticasone 125 µg	Budesonide 200 µg	Beclomethasone 100 µg	Ciclesonide 160 µg
VHC		Volumatic®	Nebuhaler®	AeroChamber®	AeroChamber ®
A. Volume percentage < 5 µm in delivered dose (%)					
from pMDI		76.69	83.11	95.90	97.50
from VHC	primed	86.46	86.17	100	100
	new	82.51	86.98	100	100
B. Volume percentage < 1.1 µm in delivered dose (%)					
from pMDI		0.25	4.12	18.87	17.14
from VHC	primed	0.58	10.35	27.07	14.18
	new	0.96	10.38	30.91	18.59
C. Volume percentage 1.0–3.0 µm in delivered dose (%)					
from pMDI		42.92	50.91	62.10	67.32
from VHC	primed	49.86	50.53	63.17	69.95
	new	47.90	50.53	62.27	69.09

Table 4.3 *Particle size fractions from pMDI and from pMDI-VHC combinations*
Comparison of particle size fractions in delivered dose from pMDI with fully primed VHC and with new (unwashed, non-primed) VHC at medium humidity. Results are expressed as a mean of 3-5 doses for pMDI and 10-25 doses for pMDI-VHC combinations (at 10 L/min). Primed VHCs compared with unwashed, non-primed VHCs did not differ from each other regarding volume percentages within delivered dose.

EFFECT OF INSPIRATORY FLOW RATE AND LAG TIME

The inspiratory flow rate did not significantly change the volume median diameter (VMD) of the aerosols *(Figure 4.2)*, and neither did the lag time between the release of aerosol in the VHC and the start of suction. The overall mean reduction in VMD after 20 seconds was less than 5% *(Figure 4.3)*.

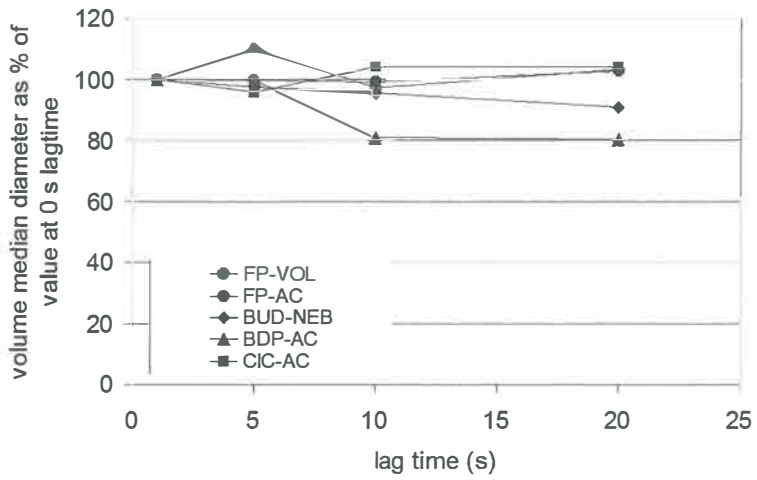

Figure 4.3 *Relative changes in the volume median diameters of the aerosols from VHCs as function of the lag time between firing a dose and aerosol suction from the VHCs at a constant flow rate of 10 L/min (5 s); testing at medium relative humidity. All data points (mean of 2 series of 5 subsequent doses taken from two different pMDIs for t > 1 second) are expressed as a percentage of the volume median diameter obtained after 1 second lag time.*

The mean reduction in delivered dose after 10 seconds lag time compared to 1 second lag time was 20.1%. This reduction was 12.7% for ciclesonide + AeroChamber®, 15.4% for fluticasone + Volumatic®, 15.8% for beclomethasone + AeroChamber®, 24.7% for budesonide + AeroChamber® and 32.7% for fluticasone + AeroChamber®. The mean reduction increased to on average 30% when a longer lag time of 20 seconds was applied *(Figure 4.4)*.

PRIMING AND WASHING OF THE VHC

Priming was complete after firing at least 10 doses into the AeroChamber® and this was the same for all three pMDIs tested (data not shown). The experiments were conducted at low humidity, under which conditions the effect of priming and washing on the delivered dose is highest. Priming after washing in a soap solution and drip drying had no additional effect.

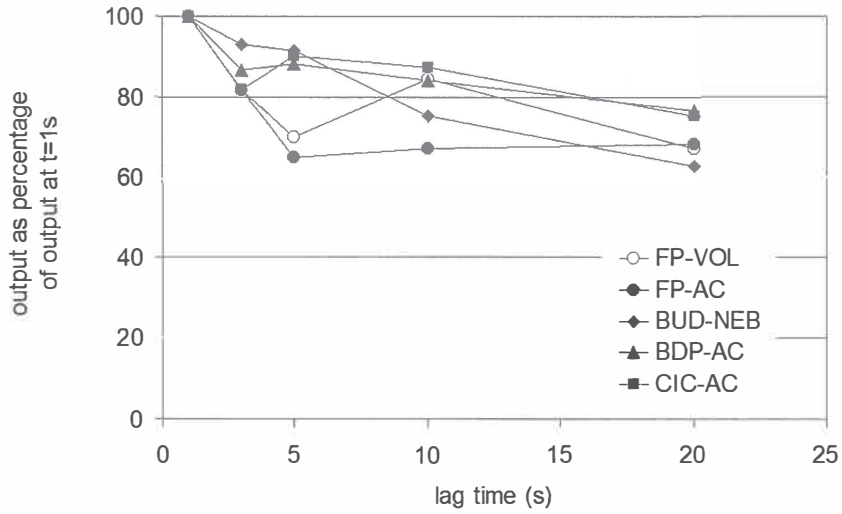

Figure 4.4 *Output from VHCs after increasing lag time, expressed as percentage of dose output after a lag time of 1 second between firing a dose and start of suction.*

DISCUSSION

The major finding of this study is that a higher relative air humidity increases the drug output from fully primed and washed VHCs, even up to a double dose difference for the fluticasone + Volumatic® combination *(Figure 4.1, Table 4.2)*. The particle size distribution does not change with increasing humidity, thus a higher output translates directly to a higher fine particle dose. Higher humidity also increases the output from the new antistatic AeroChamber®.

A second important finding of our study is that the fine particle dose delivered from fully primed VHCs at medium relative humidity may be as low as only 44% to 60% of the FPD directly from the pMDI without VHC *(Table 4.4)*. This corresponds to losses in the VHC of FPD between 40% and 56%. At low humidity these losses are even considerably higher. Low humidity frequently occurs in daily life and our data suggest that the resulting lower drug dose output from VHCs can be overcome by inhaling medication in a moist environment, like in the bathroom. Finally, our results indicate that VHCs retain particles of all sizes and not exclusively the largest particles, although the volume median diameter decreases slightly for most combinations compared to that in the aerosol directly from the pMDI *(Figure 4.2)*. The flow rate has no significant influence on the volume median diameters of ICS from VHCs *(Figure 4.2)*.

We found that VHC output increases when humidity is higher. This novel finding seems in contradiction with former studies that showed lower delivered doses to the lung with higher humidity.[23-25] However, these studies were performed with experimental setups for mechanically ventilated patients and included heating and humidifying of air (up to 100% relative humidity). Furthermore, tubes with narrow internal diameters, as well as Y-pieces and T-junctions were used to split and mix air streams by compression and suction. In our study, we collected the aerosol on a filter immediately downstream of

the mouthpiece and also measured PSD close to the mouthpiece of the VHC. We regard droplet growth as an unlikely explanation for the higher VHC output at high humidity as this would not influence the drug dose measured on the filters. Neither did we find a shift in the particle size distribution towards larger droplets at higher air humidity.

Where we found losses in the VHC between 40 and 56% at medium humidity, and even higher losses at low humidity, other authors have shown reductions between 0 and 53 % of the label claim.[26-30] In these studies the definition of fine particle fractions varied between particles of 1 to 5 µm to 'smaller than' 4.7- 5.8 µm. In our study we defined fine particles as particles from 1.0-3.0 µm, as these are the most relevant for peripheral lung deposition. Furthermore, in some other studies the effect of relative air humidity was not taken into account or reported.

The 20% reduction in dose output from a VHC after 10 s in our study was substantially less than the 50% reduction described in an earlier study.[31] This discrepancy may be due to other conditions during testing, such as humidity, which was not reported in the latter study. In our study, we did not find a gradual decrease in output as function of the lag time *(Figure 4.4)*. This may be caused by the large variation in delivered dose from the same pMDI as well as the inter-device variation as previously described.[21]
A lag time of 20 s between firing and inhalation of a dose resulted in a mean output reduction of 30% for all pMDI-VHC combinations tested. For fluticasone and budesonide with volume median diameters (VMD) of 3.5 and 3.0 µm from the pMDI respectively, aerosol losses in the AeroChamber® were higher (30-40%) with 20 s lag time than for beclomethasone and ciclesonide with VMDs of 1.9 µm (20-30%) respectively *(Figure 4.4)*.
The higher retention for aerosols with larger VMDs in the same VHC within the same lag time may be caused by sedimentation. However, the mean VMD decreased less than 5% after 20 s lag time and the reduction in delivered dose of fluticasone after a 20-second lag time was the same for the large Volumatic® and the small AeroChamber® *(Figure 4.3)*. We hypothesise that the sedimentation of larger droplets keeps the entire aerosol in motion thereby leading to sedimentation deposition of smaller particles as well.

Pre-treating VHCs by washing them in mild ionic detergent, followed by drying to the air has been presented as an effective, economical alternative to priming with medication.[19,32] This study underscores the usefulness of washing instead of priming since we found that priming with ICS after washing had no additional effect on reduction of VHC losses.

We showed that VHC retention may be considerable and is not exclusively confined to large particles of ICS in our study. In general, we hypothesize that the reasons for losses of drug in the VHC are sedimentation (caused by gravity), impaction (influenced by the high initial plume velocity as well as during suction from the VHC), particle-wall contact by aerosol circulation in the VHC and/or electrostatic charge (influenced by VHC material, VHC preparation such as priming or washing, relative air humidity and chemical properties of the drug formulation). The increasing fine particle output with increasing humidity is also true for the new anti-static AeroChamber Plus Flow-Vu®.

Why is a large fine particle fraction important? As peripheral airway inflammation is central in asthma and the highest density of steroid receptors in the lung is found in the small airways, a higher total and

peripheral lung deposition of ICS most likely will improve asthma treatment.[1,2,8,33,19] Small articles in a narrow size distribution (1-3 µm) result in a higher total and more homogeneous lung deposition than a wider fraction of particles 1-5 µm.[13,34,35] Particles < 1 µm are mainly exhaled, whereas more than 30% of the particles > 3 µm may be deposited in the oropharynx.[13]
The higher lung deposition resulting from a larger mass fraction of fine particles when VHCs are used at high humidity may improve lung function [11,12,36], quality of life [9], and asthma control.[10]

Taken together, low air humidity has a relevant and significant negative effect on fine particle output of a (fully primed and/or washed or antistatic) VHC. In both dry cold and dry hot climates, the efficacy of the therapy may be improved by administrating the medication in a moist environment (e.g. a bathroom). This is as effective as doubling the dose, and much cheaper. Adding a VHC to a pMDI reduces both total and fine particle dose delivered, but not specifically the largest particles only. We recommend a careful selection of pMDI-VHC combinations when designing new clinical studies and to record humidity as well. In general, unregistered pMDI-VHC combinations should not be used without previous (in vitro) investigation of delivered fine particle doses. The highest fine particle dose output from VHCs obtained in our study was with beclomethasone and ciclesonide, both used with the AeroChamber®.

ACKNOWLEDGMENTS

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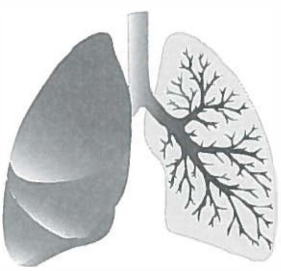
CHAPTER 5



Inhaled medication and inhalation devices for lung disease in patients with cystic fibrosis:

Which areas to target?
Which particle size?
Which device to use?

Bart L. Rottier
Anne H. de Boer
Eric J. Duiverman

J Cyst Fibros 2010;9(4):296-7

Sir,

We have read the European consensus article about inhaled medication and inhalation devices in CF therapy with great interest and would like to congratulate the authors on a great achievement.[1] The aim of inhaled antibiotic therapy in CF is to reach the best clinical response with a minimum of side effects. On three important topics regarding inhaled antibiotics no recommendations were made. With this letter we would like to put these issues forward as areas for future research, both bench- and bed-side, as well as to hypothesize on the answers.

WHICH AREAS TO TARGET?

Cystic fibrosis is thought to be a disease starting in the small airways on the basis of infant pulmonary function tests[2] and imaging studies.[3] *Pseudomonas aeruginosa* (Psa) is regarded as the most important pathogen in adult CF patients. A recent study demonstrates that Psa is present in both conductive and respiratory airways.[4] Psa is very capable to develop resistance to virtually all anti-pseudomonal agents through the selection of genetic mutations.[1]
Aminoglycosides are the most frequently used inhaled antibiotics in CF lung disease and they need high peak levels (far above the Minimal Inhibitory Concentration, MIC) to make use of the reported post-antibiotic effect of this class of drugs. Efficacy of aminoglycosides is predicted best by the peak concentration (C_{max}) to MIC ratio[5,6] and a C_{max}/MIC ratio greater than 10 is advised. A concentration gradient can be a risk for resistance development and the lowest drug concentration obtained in the lungs must be sufficiently high above MIC. According to criteria from the European Society of Clinical Microbiology and Infectious Diseases (EUCAST, www.eucast.org) Pa strains with MIC < 2 mg/L for tobramycin are considered sensitive to this type of antibiotic. Thus, to obtain an effective therapy, based on MIC and advised C_{max}/MIC ratio, tobramycin concentrations of > 20 mg/L in all airways may be necessary.

WHICH PARTICLE SIZE TO DELIVER?

In patients with stable asthma, Usmani et al. showed in a radio-isotope study that about 50% (+/– 5%) of the real dose of monodisperse particles in the size range between 1.5 and 6 micron can be deposited in the lungs. These deposition fractions were reached with a slow inhalation (mean of 30.8 L/min), followed by a breathhold period.[7] The other 50% of the dose is not deposited in the lungs, but either predominantly exhaled (especially the smaller particles) or deposited in the oropharynx. There is an increase in peripheral lung deposition from 10 to 25% of the real dose for particles with decreasing particle size from 6, to 1.5 µm. So, smaller particles can give considerably higher peripheral lung deposition which is in agreement with prediction from computational lung deposition studies.

Airway generations (gens) 17 to 23, frequently referred to as the respiratory airways, contribute more than 95% to the total airway surface area. In contrast, the transitional airways (gens 12–16) add only 4% and the conducting airways (gens 1–11) cover less than 1% to the total area.[8] Although the definitions used by Usmani et al. in their study for central, intermediate and peripheral airways are not completely comparable to these definitions for conducting, transitional and respiratory airways, it is clear that differences in the mass median aerodynamic diameter (MMAD) result in extreme differences between drug concentrations in different lung regions. Only approx. 25% of the real dose of 1.5 µm particles deposits on 95% of the total lung surface area (in the peripheral lung) versus 31% of the real dose on the complementary 5% of the total surface area (in the central and intermediate lung). This yields a difference in concentration almost by a factor 25. For larger particles or higher flow rates, the differences are even greater. A fall in concentration from upper to lower lung is therefore inevitable, but particles in the aerodynamic size range

between 1 and 2 µm yield clearly more homogeneous drug concentrations within the whole lung than particles in the size range between 2 and 4 µm, or even larger. Therefore, fine particles (1–2 µm) seem preferable for design of future delivery devices for antibiotics in CF, whereas they should be inhaled with a slow inhalation manoeuvre (inspiratory peak flow rate ≥ 30 L/min).

WHICH DEVICE TO USE?

On the basis of the small airway involvement in CF and the exponentially increasing surface area of the airways towards the alveoli, the peripheral airways should be targeted for inhaled antibiotics. Targeting should be with small particles (1–2 µm) and slow inhalation (30 L/min). The aerosol administration devices for antibiotics in CF should be selected with the objective to fulfil these requirements.

The authors describe studies with various administration devices in respect of total lung dose and mention values ranging from 3 to 73%.[1] Differences in the site of deposition may be expected from using the Pari LC ® Plus with a 'suitable' compressor for TOBI® (Pari TurboBoy® N) or the eFlow ® rapid. Under laboratory conditions (suction rate 30 L/min), the MMAD's from these devices are 2.8 and 3.5 µm respectively.[9] Also the subfractions of fine particles (particularly 1–2 micron) are different (22.6 and 14.4% of the emitted dose at 30 L/min respectively). This will result in a different distribution over the airways (even if total lung deposition is the same) from both devices.[5] A larger difference may result when the average inspiratory flow rate through the eFlow is higher than that through the LC Plus® (peak inspiratory flow rates may vary between 17 and 90 L/min for adult patients breathing through a nebulizer device.[10] The relevance of such variations to the efficacy of the treatment has to be investigated in clinical studies by comparison of the effect of the same drug administered with different MMAD's and different flow rates. A breathhold period after inhalation is important when using a DPI in order to achieve significant deposition by sedimentation. When the prerequisites of MMAD and flow manoeuvre are considered, both a dry powder inhaler or a nebulizer-system may be appropriate.

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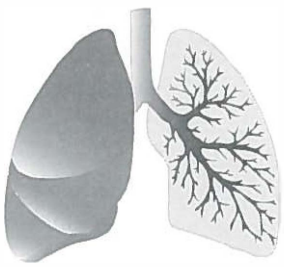
CHAPTER 6



Changes in performance of the Pari eFlow® rapid and Pari LC® Plus during 6 months use by CF patients

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Abstract

Background:

Nebulized antibiotics are important in the treatment of cystic fibrosis. The Pari eFlow® rapid with vibrating mesh is often used off-label for the administration of tobramycin (TOBI®) because of a reduced nebulization time and easier handling compared to a classic nebulizer–compressor combination. Mesh technology may be vulnerable, however. Therefore, we investigated particle size distribution and output as well as changes in the performance of the eFlow before and after 6 months of use, in comparison with the Pari LC® Plus nebulizer plus Turboboy® compressor.

Methods:

Size distributions in the aerosols and nebulization times for TOBI® were measured with laser diffraction technique; delivered doses by weighing.

Results:

New eFlows produce considerably larger droplets (X_{50}= 3.5 µm) from TOBI® than new LC Plus nebulizers (X_{50}= 2.8 µm). After use, the X_{50} increases for both systems (to 3.7 and 3.3 µm, respectively). The relative span of the size distribution $\{(X_{90}-X_{10})/X_{50}\}$ changes from 1.26 to 1.28 µm for eFlow and from 2.19 to 2.45 µm for LC Plus. The total nebulization time doubles for LC Plus, whereas in 51% of all experiments the eFlow switched off after 10 min, resulting in incomplete dose delivery. For the eFlow, changes during use are related to clogging of orifices. Once being clogged, only replacement of the mesh restores the original performance.

Conclusions:

New eFlows produce larger droplets and in a narrower size range compared to new LC Plus nebulizers for TOBI®, and therefore both devices are not equivalent. Theoretically a larger portion of the aerosol from eFlow is likely to be deposited in the upper airways. The performance of both tested nebulizers decreases after 6 months of use. For the eFlow, timely replacement of the mesh is necessary. These in vitro results underscore the importance of registration studies of new drug-device combinations.

INTRODUCTION

In patients with cystic fibrosis (CF) inhaled tobramycin is widely used to suppress *Pseudomonas aeruginosa* with the aim to preserve lungfunction and to prevent pulmonary exacerbations.[1-3] A special tobramycin solution for inhalation (TSI: TOBI®) was developed for use with the Pari LC® Plus (LC Plus) with an appropriate compressor.[4] Nearly a decade after the FDA approved TOBI® with a DeVilbiss Pulmo-Aide® compressor (1997), the new Pari eFow® rapid (eFlow) with vibrating mesh technology[5] was cleared for the U.S. (2004) and the European markets (2005). Immediately afterward, the registered eFlow was in use for the aerosolization of TOBI® in many different countries, although the combination of drug and device has not officially been approved and registered. The manufacturer of both devices showed in an in vitro deposition study (with the Andersen impactor) that LC Plus and eFlow produce aerosols with different size distributions from this drug solution.[6] Reduced nebulization time and ease of handling, both increasing patient compliance, were important stimuli for this off-label use. The difference in median droplet size and size distri-bution between LC Plus and eFlow may have consequences for the site of deposition and safety, however. Although no firm recommendation for the target area can be found in the literature[7], it has been shown that most adult CF patients have recurrent upper airway infection with *Pseudomonas aeruginosa* from which colonization of lower airways occurs[8] up to (and including) the alveoli.[9,10] In addition to the difference in droplet size distribution between the LC Plus and eFlow, concern has been raised about the durability of the eFlow in daily practice due to expected vulnerability of the mesh, particularly in relation to cleaning and sterilization procedures. Therefore, the aim of this study was to verify the difference in performance of new devices as presented by the manufacturer and to investigate whether changes occur in the performance of both devices in daily practice (6-month therapy) when being used for the administration of TOBI®. Such changes could have relevant implications for lung deposition, and may further increase the difference between the aerosols from the eFlow and the LC Plus.
Laser diffraction technique was chosen because laser diffraction data are already available for the LC Plus from a previous study.[11] To make the results more comparable, used eFlow and LC Plus nebulizers were collected from the same group of patients.

MATERIALS AND METHODS

Study design

Fifteen adult cystic fibrosis patients in The Netherlands using a nebulizer for the administration of TOBI® were advised by their physician to change over to the eFlow for the administration of TOBI® and asked to volunteer in the study. The costs for these new devices were refunded by Chiron (now Novartis, East Hanover, NJ). The 10 patients who used a LC Plus nebulizer until then, handed it in for performance testing. They were given appropriate instructions on cleaning, and provided with a disinfection device. After 6 months of use (three cycles of 28 days TOBI® treatment) they handed in their eFlow for performance testing with a questionnaire regarding experiences, cleaning procedures, and other drugs nebulized with the device.

On returning their used eFlow (series 1), all patients received a new (substitute) device, also financially provided by Novartis. Collected (used) eFlows were checked on proper cleaning of the devices before starting the performance testing. Because some meshes appeared to be visibly polluted, it was decided to extend the study: all patients were asked to hand in their substitute eFlows for performance testing again after another 6 months use, and 8 out of 15 patients agreed to do so (series 2). Before the patients started using their substitute devices, even more emphasis was put on correct cleaning and handling compared to the first series.

The patients participating in this study were advised by their physicians to change over to the eFlow for therapy (compliance) related reasons and not asked to change over for this performance testing study. Therefore, this study is considered an observational study without intervention, and approval from an ethical committee according to the the (Dutch) Law on Medical Scientific Research involving Human Beings (WMO) was not necessary. The median period of use for the collected LC Plus nebulizers was 6 months.

MATERIALS

TOBI® ampoules, exposed to room temperature 15 min before use, were supplied by Red Swan pharma logistics (Utrecht, The Netherlands). Nebulizers and Petra disinfection apparatus were supplied by Novartis. eFlows (both new and used) were tested with a Pari mouthpiece with an exhalation filter in agreement with daily practice. Two used eFlows from the first series of 15 were returned without the vibrating mesh, and were therefore excluded from the testing program. One of the visibly polluted devices from the second series was also tested after the mesh was replaced by a new one. The 10 used LC Plus nebulizers were tested in combination with a Pari TurboBoy® N compressor. This compressor from the same manufacturer complies with the specifications mentioned for the aerosolization of TOBI® (jet flow is 4 L/min). Using the TurbuBoy® N also enables comparison with data presented previously for new LC Plus nebulizers.[11] Actually, the same compressor device was used for the new and used nebulizers after it was checked so that the jet flow was still the same and meeting the specifications of the manufacturer.

MEASUREMENT OF PARTICLE SIZE DISTRIBUTION IN THE AEROSOL

Particle size distributions in the aerosols were measured with a HELOS Compact/KA laser diffraction apparatus (Sympatec, Germany) at a constant flow rate of 30 L · min^{-1} using a 100-mm lens (measuring range from 0.5 to 175 µm), an INHALER 2000 adapter, and the Fraunhofer theory for the calculations.[12] All eFlows (new and used) were tested three times, using three different TOBI® ampoules; used LC Plus nebulizers were tested in duplicate. The first test with used devices was as collected from the patients. Between the tests, the devices were cleaned according to the procedures prescribed to the patients. A series of measurements of 10 sec each was started from the beginning of the nebulization process with interval times of 20 sec. Data from the eFlows were collected until the devices stopped nebulization (on detection of a minimum liquid level, or after 10 min). Measurement of LC Plus was continued into the phase of sputtering, but the data obtained during sputtering were omitted from processing. Cumulative volume distribution curves (as function of diameter) were averaged per ampoule first, and next, the mean of three ampoules per device was calculated in order to obtain an overall mean size distribution per device.

Data are presented as cumulative volume distribution curves *(Figure 6.1)*, or as characteristic values (X_{10}; X_{50} = VMD and X_{90}) derived from these curves *(Figure 6.2* and *Table 6.1)*. The variations given in *Table 6.1* and spread bars in *Figure 6.2* represent the highest and lowest X50 values obtained within all individual series (10 sec measurements) per device. The spread bars in *Figure 6.1* represent the standard deviation for the averaged values per time interval. Because the cumulative volume distribution curves were not log-normal, geometric standard deviations (GSD's) could not be calculated. Instead, relative spans (RS) of the size distributions $\{RS = (X_{90}-X_{10})/X_{50}\}$ are presented *(Table 6.1)*. The mouthpieces of the nebulizers were positioned less than 5 mm from the laserbeam to exclude droplet evaporation. Unpaired Student t-tests were performed for statistical analysis.

NEBULIZATION TIMES AND DELIVERED DOSES

Total nebulization times (until switching off for the eFlow and the start of sputtering for the LC Plus, respectively) were recorded for individual ampoules and averaged per device. Delivered doses (in mg drug solution) were calculated from differences in weight before and after nebulization. Delivered weight may overestimate delivered drug dose because of evaporation in the LC plus nebulizer cup (not for the eFlow).[13] Mean optical concentrations in the aerosol cloud were recorded to detect changes in the output rate, indicative for dry running of the LC Plus nebulizers and severity of clogging of the vibrating meshes in the eFlows.

RESULTS

Particle size distributions in the aerosols

Figure 6.1 shows the volume median droplet diameters (VMD = X50) with standard deviations and optical concentrations (Copt) in the aerosols from new nebulizers as function of the nebulization time. The curves represent the mean of five devices. For both types of nebulizers, the droplet size distribution is constant except for the first 10 sec of nebulization and after the optical concentration starts to decrease significantly. *Table 6.1* compares the overall mean data for new and used devices and shows that the volume median diameter for TOBI® from a new eFlow is approximately 24% higher than that from a new LC Plus ($p < 0.05$). However, the RS of the size distribution is narrower for the eFlow than for the LC Plus ($p < 0.05$).

The interdevice variation before and after use is shown in *Figures 6.2* A (for eFlow) and B (for LC Plus). The spread bars in *Figure 6.2* A and B indicate the maximum and minimum values obtained within the three ampoules. The relative standard deviation (RSD in X50) was found to increase from 2.8% for new eFlows to 6.7% (series 1) and 8.4% (series 2) for used devices and from 2.9% (new) to 11.4% (used) for LC PLus, respectively (all $p < 0.05$). Table I shows that overall mean values for X50 and RS both increase for eFlow (as well as for LC Plus) during use and both increases are significant ($p < 0,05$).

Figure 6.3 A shows the array of round orifices of an unused vibrating mesh. For polluted meshes *(Figure 6.3* B and C*)*, orifices were either still more or less completely open, or practically totally clogged (as in *Figure 6.3* C*)*. The fraction of more or less totally clogged orifices in a severely polluted mesh was over 50% *(Figure 6.3* B*)*.

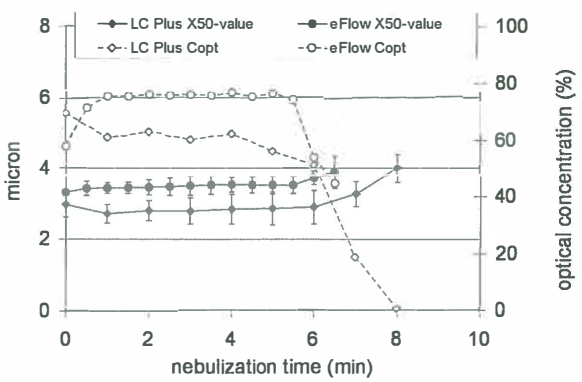


Figure 6.1 *Volume median droplet diameters (X50-values) and optical concentrations (Copt) in the aerosols from a new eFlow and a new LC Plus nebulizer (plus TurboBoy N compressor), respectively, as function of the nebulization time (mean of five devices). The spreadbars indicate the standard deviations.*

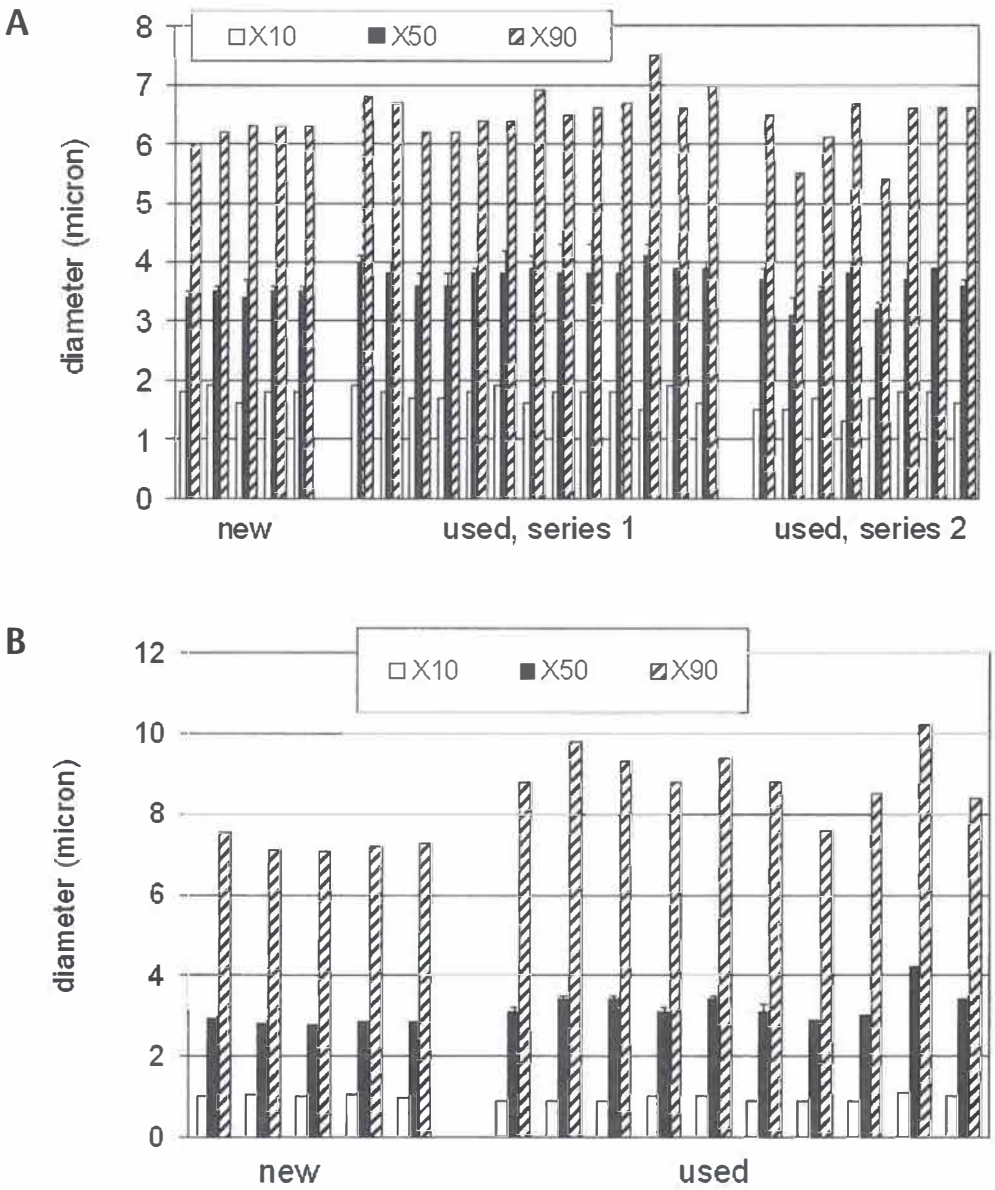
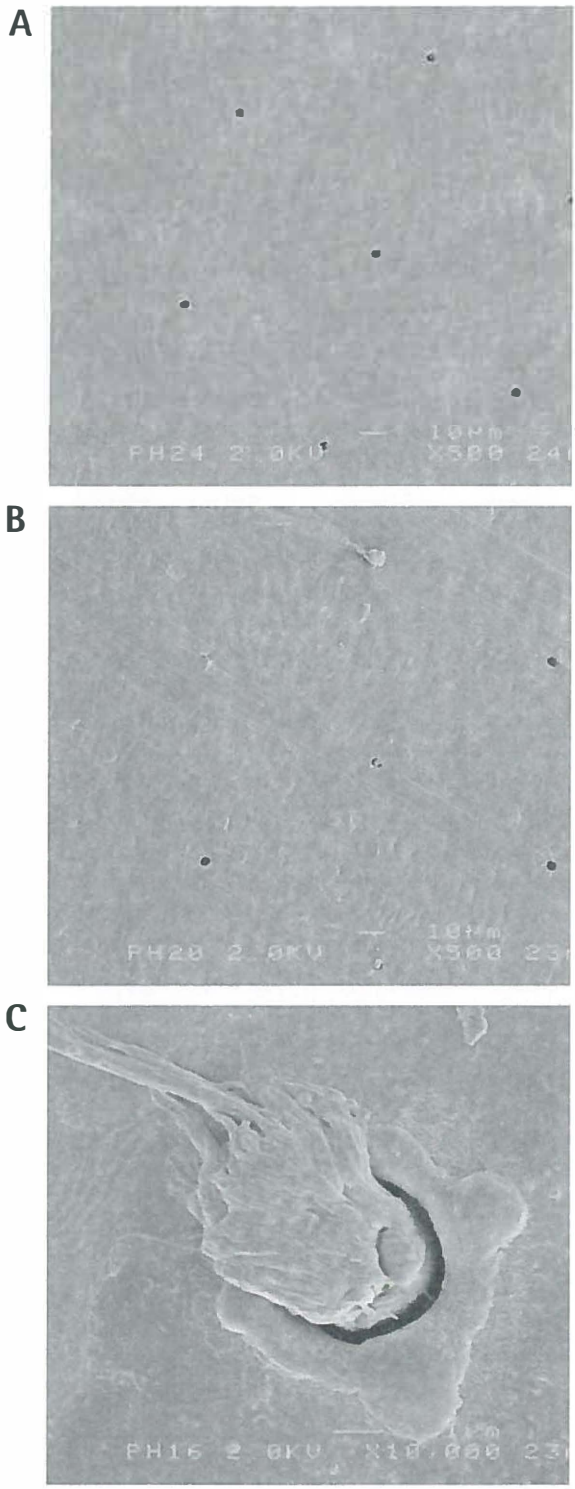


Figure 6.2 *Comparison of characteristic values (X_{10}, X_{50}, and X_{90}) from the cumulative volume distributions (as function of the diameter) for the aerosols from new and used eFlow (A) and LC Plus nebulizers (B). The spread bars (X50) indicate maximum and minimum values obtained within individual series.*



NEBULIZATION TIMES AND DELIVERED DOSES

Table 6.1 shows that the mean nebulization time for the eFlow devices increased during use from 6.7 to (on average) 8.9 min ($p < 0.05$). This increase does not reflect the dramatic difference in delivered solution weight between new and used devices, as the eFlows used in this study were programmed to switch off automatically after 10 min. It was found for the 21 used eFlows, each of them tested three times with three different ampoules (63 measurements in total), that during 32 measurements (51%) the device was switched off prematurely. This percentage was higher for series 1 (56%) than for series 2 (42%), possibly as a consequence of putting more emphasis on the need for correct cleaning when the devices of series 2 were handed to the patients. In total, eight devices switched off prematurely for all three ampoules *(see Table 6.1)*. As a consequence, part of the dose was not delivered. Actually, the delivered dose for both series of used devices varied from 1.15 (22%) to 4.21 g (79%) out of the (on average) 5.3 g TOBI® solution. For new devices, the variation was only between 3.67 (69%) and 4.02 g (76%) of the real dose. When a polluted mesh was replaced by a new one (as for one of the severely polluted devices in series 2), the nebulization time (7.5 min) and the delivered dose (3.76 g) were restored to original values.

In contrast with the nebulization times for the eFlow, these data for LC Plus depend on the inhalation manoeuvre and will be different with tidal breathing compared to a constant flow rate. This may result in a more than doubled nebulization time with (in vivo) tidal breathing. In addition, the effect of 6 month use is a twofold increase in nebulization time for LC Plus *(Table 6.1)*. The flow rate with

Figure 6.3 *Scanning electron micrographs of a new (A) and a heavily polluted used mesh (B and C) from the eFlow.*

which the aerosol is drawn from the nebulization cup determines the fraction of particles deposited on the baffle and inner walls of the cup, and thus, the reflux of drug solution. Therefore, nebulization times and delivered doses for the LC Plus in *Table 6.1* are only useful for comparative evaluation between new and used devices. Additionally, delivered drug dose cannot be derived from weight loss of the nebulizer cup because of liquid evaporation: drug concentration in the nebulizer cup during nebulization may increase up to nearly 140% of the original value.[13] It was found that total nebulization time for the LC Plus at a constant flow rate increases considerably more than that for the eFlow, because the LC Plus does not switch off after 10 min.

Device	*Volume median diameter (X_{50}) (micron)*	*Relative span of the size distribution (X_{90}-X_{10}/X_{50})*	*Delivered dose (g drug solution)*	*Total nebulization time[a] (min)*	*Average output rate (g/min)*	*Number of eFlows switched off prematurely[b]*
eFlow new	3.5 (3.3–3.7)	1.3	3.85 (3.67–4.02)	6.7 (5.5–8.0)	0.58	15/0/0
eFlow used, series 1	3.8 (3.3–4.3)	1.3	3.35 (1.65–4.21)	9.0 (5.5–10)	0.37	39/22/6
eFlow used, series 2	3.5 (2.8–4.0)	1.3	3.48 (1.15–4.09)	8.7 (6.5–10)	0.40	24/10/2
LC Plus new	2.8 (2.7–3.0)	2.2	3.44 (3.41–3.50)	6.9 (6.8–6.9)	0.50	—
LC Plus used	3.3 (2.8–4.2)	2.5	4.19 (3.61–4.44)	13.0 (8.0–20.5)	0.32	—

[a]For eFlow: time reduced due to devices switching off automatically after 10-min nebulization time. [b]The first figure indicates the total number of ampoules tested (three per device); the second figure indicates to the total number of switchings off; the third figure indicates the number of devices for which none out of three ampoules was completely nebulized.

Table 6.1 *Comparison of overall mean median diameters (X_{50}), relative spans of the size distributions {(X_{90}–X_{10})/X_{50}}, delivered doses (g drug solution), total nebulization times (min), and output rates (g drug solution per minute) for new and used eFlows and LC Plus nebulizers.*
Results between brackets are the lowest and highest values observed.

PATIENT COMPLIANCE

Although all patients in the study indicated to have cleaned their eFlow with great care and consistency (warm water detergent), most eFlows were returned with polluted meshes. The same was true for the devices of the second series, despite more emphasis on good cleaning procedures, particularly after the last use. The majority of patients (85% in series 1; 75% in series 2) also used their eFlow for the administration of other medicaments than TOBI®. They nebulized salbutamol, ipratropium, terbutalin, colistin, DNAse (Pulmozyme), acetylcistein and/or saline.

DISCUSSION

The objectives of this study were to verify differences in droplet size distribution between eFlow and LC Plus (for TOBI®) and to investigate possible changes in the performance of the vibrating mesh technology applied in the eFlow during (6 months) use in daily life (CF patients). With respect to the latter, the outcome of the study is that particularly the interdevice variation increases during use. Surprisingly, the changes in performance for the eFlow are less extreme than those concluded for the LC Plus (based on comparison of previous data for new LC Plus nebulizers and new data for used devices in this study).

This study has also confirmed that the VMD's in the aerosols from new eFlows (3.52 µm from laser diffraction analysis) and the new LC Plus nebulizers (2.84 µm) are not the same. Pari presented for TOBI® a mass median aerodynamic diameter from the Andersen cascade impactor (at 28.3 L/min) of 3.95 µm for the eFlow and 3.5 µm for the LC Plus, while using the same compressor we used in this study.[6] The differences between our study and data from Pari® cannot be explained easily. Contributing factors are that different measurement principles have been used and that different conditions such as relative air humidity may have been different. However, the difference in median diameter between the eFlow and LC Plus is consistent for both studies. The difference in median diameters between eFlow and LC Plus (by 24% in this study) raises concern about the site of deposition in the human lung, and therefore, the therapeutic effect and safety. Based on the impaction parameter ($IP = \rho.X_{50}^2.U$, where ρ is the particle density and U is the particle velocity), the difference is even 54%. In terms of fine particle fraction, the fraction <3.1 micron is 25% lower from eFlow than from LC Plus. In daily life, this difference may be even greater, because the manufacturer of TOBI® recommends using a compressor with a jet flow within the range from 4 to 6 L/min. In the present study, a compressor with a jet flow of 4 L/min was used, and from previous investigations it is known that higher jet flows result in smaller droplets.[11,14-16] Hence, based on in vitro deposition data, the eFlow does not seem to be equivalent to the LC Plus. The volume median diameters of used eFlows and LC Plus nebulizers differ less from each other (13%) than those of new devices (24%) because the increase in volume median diameter during use is higher for the LC Plus (15.5%) than that for the eFlow (5.1%).

For nebulized antibiotics a high drug concentration on the site of infection is required. As already mentioned in the introduction, *Pseudomonas* infection may involve the whole lung. Consequently, the whole lung has to be treated with the antibiotic drug. Based on the exponentially increasing inner surface area of the airways from the lobar bronchi to the alveoli, an increasing amount of drug is necessary toward the lower airways in order to obtain a more or less equal drug concentration throughout the entire lung. According to various lung models[17] the conducting airways (generally 0–11) contribute only approximately 1% to the total airway surface area, versus 4% for the transitional airways (generally 12–16) and 95% for the respiratory airways (generally 17–23). Hence, on the basis of the surface area distribution and the desired equal drug concentration, the amount of drug to be deposited in the respiratory airways needs to be 24 times higher than that in the conducting and transitional airways combined (generally 0 to 16). However, when aiming at deposition in the respiratory airways, losses occur in the preceding conducting and transitional airways. According to lung deposition models[18], these losses exceed the approximately 5% that is needed in these regions. This supports the idea that antibiotics for equal distribution over the entire lung have to exhibit the appropriate particle size for deposition in the respiratory airways, or the concentration in the deep lung will be much lower than that in the conducting and transitional airways.

From both deposition modelling[18-20] and in vivo deposition studies[21], it is known that the optimal particle size for peripheral lung deposition at moderate flow rates (30–60 L/min) is rather between 2 and 3 µm than between 3 and 4 micron. Peak inhalation flow rates during tidal breathing as measured with a pneumotachograph may be in the range of 30 to 90 L/min for CF patients.[22] Because it is known that mean flow rates are approx. 70% of peak flow rates[23], this reduces the expected range of mean flow rates through the eFlow to 20–60 L/min. As an overall conclusion, it seems rational to expect that the ratio of aerosol deposition in the conducting and transitional airways to that in the respiratory airways is higher from the eFlow than from the LC Plus. Therefore, it is recommended that a comparative in vivo deposition study is conducted to verify this expectation.

The reasons for a change in median droplet size during use of the eFlows have not been investigated. It has been observed though, that the wetting of the mesh and the adherence of expelled gas bubbles to the mesh surface change during use. This could influence the droplet formation process and the influence could depend on the mesh pollution. The size distribution in a wet aerosol is also affected by droplet coalescence and evaporation, which depend on the droplet concentration in the aerosol. For the eFlow, this droplet concentration is related to the number of open orifices. Clogging *(Figure 6.3 B)* appeared to reduce the optical concentration (Copt) from 80% for new meshes to less than 20% for strongly polluted meshes. When clogging of an orifice occurred, it seemed to be more or less complete *(Figure 6.3 B–C)*, although it is expected that also partially clogged orifices do not produce droplets because the resistance to flow through an orifice increases exponentially with its diameter. Above a certain threshold value for the resistance, no droplets will effectively be formed. This may explain why the effect of mesh pollution on output rate is much greater than that on particle size distribution in the aerosol. Despite the emphasis put on good cleaning procedures in the instructions for use, mesh pollution was visible for nearly all collected eFlows, but the devices of series 2 showed less clogging than the devices of series 1 (with only one exception). Considering that more emphasis was put on correct cleaning at the start of series 2, it must be concluded that cleaning and maintenance have a great effect on mesh pollution. In this respect, the mesh technology is vulnerable. Pollution may also have been influenced by unintended nebulization of other drug solutions than TOBI® with the same device. Cleaning of a polluted mesh during performance testing appeared hardly effective in regaining the original eFlow performance. Only in a few examples it was observed that the second and third experiment of used devices (after cleaning) yielded slightly better results than the first. However, replacement of a polluted mesh fully restored the particle size distribution and nebulization time to values for a new device. Pari® has recently developed an "easy care cleaning aid" for this purpose, but data about use in daily practice and efficacy have not been published yet. Possible reasons for differences in performance between new and used LC Plus nebulizer cups have been explained and discussed before.[11]

For the eFlow, the increase in nebulization time eventually leads to automatic switching off after 10 min. This implies that part of the dose is not administered to the patient and this has resulted in extreme variation in delivered dose in this study *(Table 6.1)*. In a recent personal communication with Pari we were informed that the time for automatic switch-off will therefore be prolonged to 20 min. This change in technical specification may indeed decrease the variation in delivered dose, but it will also increase the necessity to measure the nebulization time because of the observed increase in inter device variation due to mesh pollution. Nebulization of other drug solutions than TOBI® with the eFlow should be strongly dissuaded. Although we could not find clear correlations between the degree of clogging and the type of drug solutions nebulized in this study, neither with the cleaning methods applied, it may well be that mesh pollution is influenced by these parameters. Apart from possible consequences for mesh pollution, the droplet size distribution may vary with the properties of the drug solution.[14] Moreover, not all drug solutions are supplied in the same large volume as TOBI®. Considering the high residual volumes left in the medicament chamber after the eFlow is switched off (upon detection of minimal level), the delivered dose may vary quite substantially with the volume of the drug solution inserted in the medicament reservoir.

CONCLUSIONS

LC Plus nebulizers and eFlows produce aerosols with different droplet size distributions for TOBI®: the median droplet diameter from a new eFlow is 24% larger (fine particle fraction < 3.1 micron is 25% lower) and the difference between the devices becomes even greater when a compressor with a higher jet flow is used. In addition, the size distribution is narrower from the eFlow. This is likely to have consequences for the drug deposition in the respiratory tract and the finding underscores the importance of extensive in vitro (and in vivo) testing before selecting formulation–nebulizer combinations for clinical use.[24,25] During use, median droplet diameters appear to slightly increase for both devices, but the observed increase was somewhat higher for the LC Plus than for the eFlow. Therefore, the aerosols from used devices are more comparable than those produced by new devices. During use the interdevice variation and nebulization time increased for the eFlow, which seems most likely to be the result of mesh pollution (clogging of orifices). This occurred despite good emphasis on the cleaning instructions, and there may have been an influence on clogging from nebulizing other drug solutions than TOBI® with the same device, like Pulmozyme. Mesh pollution changed the output rate quite dramatically, and the decreased output rate in combination with automatic switch-off after 10-min nebulization time resulted in an unacceptable variation in delivered dose. Therefore, timely replacement of polluted meshes is mandatory.

These in vitro data suggest that the eFlow is not equivalent to the LC Plus for TOBI® administration, but a clinical effectiveness study (including assessment of adhearance) should be performed to confirm that conclusion.

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ABBREVIATIONS AND SYMBOLS

C_{opt}	Optical concentration in the aerosol. Copt is a measure for the light extinction, which is influenced by the droplet density and the droplet size distribution in the aerosol
GSD	Geometric standard deviation (for log-normal distributions)
MMAD	Mass median aerodynamic diameter
RS	Relative span of the size distribution; ratio of (X_{90}–X_{10}) to X_{50} (alternative for geometric standard deviation for skewed distributions)
VMD	Volume median diameter
X_{10}	Diameter derived from the cumulative volume distribution curve: 10% of the volume is in particles smaller than X_{10} (micron)
X_{50}	Diameter derived from the cumulative volume distribution curve: 50% of the volume is in particles smaller than X_{50} (micron)
X_{90}	Diameter derived from the cumulative volume distribution curve: 90% of the volume is in particles smaller than X_{90} (micron)

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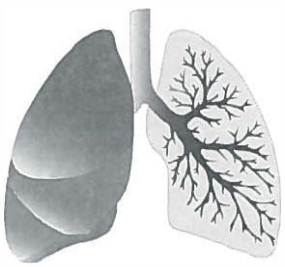
CHAPTER 7



Do the aerosol properties of inhaled antibiotics used in Cystic Fibrosis vary between drug solutions and jet nebulizers?

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Francesca Scotti
Paul Hagedoorn
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Eric J. Duiverman
Anne H. de Boer

submitted

Abstract

Background:

Nebulized tobramycin and colistin are widely used against *Pseudomonas aeruginosa* (Psa) in the airways of cystic fibrosis patients. Psa is present in both large and small airways and thus, the entire lung needs to be targeted. Effective targeting depends on drug mass output, particle size distribution and inhalation manoeuvre. Therefore, antibiotic formulation, nebulizer, jet pressure and inspiratory flow rate may all influence the particle size distribution in the aerosol but to which extent is unknown. In this study the aerosol properties of four antibiotic products using four nebulizer systems at different jet pressures were assessed

Methods:

Two available tobramycin solutions for inhalation (TOBI®, Bramitob®) and two colistin brands (Colistin®, Promixin®) restituted as solution were tested with Pari LC® plus, Pari LC® sprint, Pari LC® Sprint Star and Ventstream® with jet pressures of 1, 1.5, 2 and 2.5 bar. Differences in the particle size distribution of the aerosols were measured with laser diffraction technique.

Results:

All four nebulizers deliver finer aerosols when the jet pressure or the suction flow rate is increased. Volume median diameters for TOBI at 2.5 bar jet pressure and suction flow rate 20 L/min from LC® Sprint Star (1.82 µm) and Ventstream® (1.73 µm) are considerably smaller than those from LC® Plus (2.21 µm) and LC® Sprint (2.15 µm). There is no difference in particle size distribution between all four drug solutions from the same nebulizer type at the same jet pressure.

Conclusions:

For fine aerosols, a jet pressure of 2.5 bar is optimal and Ventstream® results in smallest VMD. Especially for less powerful compressors, the choice of a nebulizer is important. Regarding particle size distributions, the tested tobramycin and colistin solutions are the same from the same nebulizer used with the same jet pressure.

INTRODUCTION

Lung disease remains the major cause of morbidity and mortality in cystic fibrosis (CF). The airways of 80% of patients with cystic fibrosis (CF) will eventually become permanently infected ('colonised') with *Pseudomonas aeruginosa* (Psa).[1] Chronic infection with Psa is associated with an accelerated decline of lung function and should therefore be treated.[2,3] The antibiotics colisthimethate sodium, tobramycin and more recently, aztreonam have all been used as inhaled antibiotics to suppress Psa in the CF airways.[1,4,5] Both tobramycin and colisthimethate sodium have also been successfully used in an effort to eradicate Psa from the airways, at least temporarily, in cases of intermittent Psa colonization.[1,6,7] As Psa is present in all airway generations, including the peripheral airways up to the alveoli, the target area for inhaled antibiotics should therefore be the whole lung.[8] A droplet size in the range of 1-3 μm is expected to be the most effective as this size range will give the most homogeneous distribution over the airway tree when inhaled with a relatively slow inspiratory flow rate (30 L/min).[9] In vivo deposition studies with particles in this size range showed that approximately a half to one third of the lung dose is deposited in the peripheral lung and the remaining part in the central and intermediate lung.[10] With the exponentially increasing airway surface area from the central lung towards the alveoli, this will result in a comparable decrease in antibiotic concentration from the central lung to the peripheral airways.[10] As the efficacy of aminoglycosides depends on a high peak level, preferably a factor 10 above the Minimal Inhibitory Concentration (MIC) against Psa[11,12], high peripheral deposition is even more important.

Tobramycin solution for inhalation (TOBI®: 300 mg/5 ml) is registered in combination with the Pari LC® Plus reusable jet nebulizer and a suitable compressor resulting in a flow rate of 4-6 L/min. Another tobramycin solution is Bramitob® (300 mg/4 ml), also registered in combination with a Pari LC® Plus reusable nebulizer and the Pari Turboboy N® compressor.[13] According to registration texts, colisthimethate sodium is available as Promixin®, to be nebulized with a 'suitable compressor-nebulizer system' and Colistin®, registered with 'a compressor providing a flow over the nebulizer of 6-8 L/min' or the eFlow rapid®. Although drugs may be licensed with certain systems, new nebulizers are commonly used without registration and patients may switch to another drug without switching to the prescribed nebulizer system for that new drug. The reasons for doctors or patients to use other than registered combinations may be expected increased adherence and improved peripheral airway deposition and thereby, increased effectiveness[14]; shorter administration time[15], portability and low noise level or a combination of all these. However, switching from nebulizer system may lead to the generation of larger droplets with decreased peripheral airway deposition as consequence.[16]

The in vitro performance of different nebulizers with regard to particle size distribution (PSD) in combination with the two available tobramicin and colisthimethate sodium inhalation solutions for off-label nebulizers has not been systematically reported. Therefore, the first aim of this study was to investigate the effect of nebulizer and compressor choice and use on the aerosol properties of the four drug solutions. If different, a switch from one combination to the other may have consequences for the lung deposition of the drug. The outcome of the study may then guide clinicians in deciding which systems and antibiotics can be used and interchanged freely when used with same breathing characteristics.
A second aim was to find the most effective nebulizer-pressure combination based on the smallest volume median diameter in the aerosol.

MATERIALS AND METHODS

Materials

The jet nebulizers used in this study were Ventstream (Philips Respironics, UK, supplied by Romedic BV, The Netherlands) , LC® Plus (Pari GmbH, Germany, supplied by Romedic BV, The Netherlands), LC® Sprint and LC® Sprint Star (both Pari GmbH, Starnberg, Germany, supplied by Pari Benelux, Brussels). All nebulizer cups were washed before use to remove fatty coatings. Multiple nebulizer cups of the same type were used after it was checked with water that they produced comparable aerosols under the same circumstances. For the LC family mouthpieces exist with and without exhalation valve. After it was checked that the presence of the valve does not influence the aerosol properties, only mouthpieces without valve were used to make sure that no false air along this valve contributes to total suction flow rate. Parts of the nebulizer cups were marked to assist reproducible re-assembly for further testing after cleaning. Compressors used in the study were TurboBoy N® (Pari GmbH, Starnberg, Germany) and CR60 (Philips Respironics, supplied by Romedic, the Netherlands).

TOBI® (300 mg/5ml) (supplied by Novartis, Arnhem, The Netherlands and Bramitob® vials (supplied by Chiesi) were stored in the refrigerator and exposed to room temperature 15 minutes before use. TOBI®contains 300 mg tobramycin and 11,25 mg sodium chloride dissolved in 5 ml water filled in ampoules. The solution is adjusted to pH 6. Bramitob® (300 mg/4ml) is a solution of tobramycin in water without additives.

Colistin® (supplied by Grünenthal GmbH, Aachen, Germany), colistimethate sodium, is delivered as a dry powder in amounts of 80 mg. Each dose is accompanied by a vial with a 3 ml 0.9% aqueous sodium chloride solution to dissolve the powder.

Promixin® (supplied by Romedic, The Netherlands) is colistimethate sodium, also supplied as 80 mg powder doses. According to the instruction a dose of Promixin® was dissolved in demineralized water.

Methods and study design

A constant jet pressure from the pressure mains of 1.0, 1.5, 2.0 and 2.5 bar was used for each nebulizer *(Figure 7.1)*. Pressures were controlled with a pressure regulator having a digital manometer for accurate and reproducible adjustment (Rototherm 0-6 bar, the British Rototherm Co. Ltd, UK). A constant pressure enables to test nebulizer performance over a wider range of values than with compressors. As nebulizers have different air flow resistances, jet pressures from the same compressor are different. This makes comparison of nebulizer performance at the same jet pressure generated with compressors difficult. Furthermore, pressurised air from the mains excludes a decrease in compressor performance as a possible cause for changes in particle size distribution over time. In order to rule out a potential difference between particle size distribution from pulsatile air pressure generated with compressors and continuous air flow from the mains, the performance of the same nebulizer cups with both air sources was compared at precisely the same pressures. For this comparison, the mean values of the pulsatile jet pressures generated by the Turbo Boy N® and CR 60® compressors in combination with the LC® Plus and Ventstream® nebulizers were measured. Next, exactly the same pressures were adjusted with a constant air pressure from the mains for these nebulizers.

Compressors (pulsating flow)	**Mains (constant flow)**
• Turboboy® N	• 1,0 bar
• CR 60®	• 1,5 bar
	• 2,0 bar
	• 2,5 bar
Nebulizers:	**Nebulizers:**
• PARI LC® Plus	• PARI LC® Plus
• Ventstream®	• PARI LC® Sprint
	• PARI LC® Sprint Star
	• Ventstream®
Inhalation flowrate and drug	**Inhalation flowrate and drugs**
• 20 l/min water	• 10l/min: TOBI®
• 20 l/min TOBI®	• 20l/min: TOBI®, Bramitob®, Colistin® and Promixin®
	• 30l/min: TOBI®
	• 20l/min: water measurements

Figure 7.1 *Experimental set up*

Measument of the particle size distributions in the aerosol

Particle size distributions were measured with laser diffraction technique (HELOS BF MAGIC) using an inhaler adapter (INHALER 2000), both from Sympatec, Clausthal-Zellerfeld, Germany. A 100mm lens was used with a measuring range of 0.5 to 175 µm and the Fraunhofer approximation theory for computation of the size distributions from the complex diffraction patterns. For the theoretical background regarding the Fraunhofer and Mie theory a reference is made to the literature.[17] Briefly, the Mie theory would be more appropriate for assessment of the aerodynamic size distributions, but using this theory requires exact knowledge of the complex refractive index of the drug solutions. However, this refractive index changes during the nebulization process as the drug concentration increases due to evaporation and therefore, the Mie theory can not be applied properly. Further, as the study is a comparative evaluation between different drug solutions, nebulizers and conditions, differences in size distributions between different experiments are more relevant than absolute aerodynamic values. During nebulization of tobramycin solutions, size distribution measurements with a duration of 10 seconds were repeated with thirty seconds interval after an initial lag time of 10 s to stabilise the nebulizer performance. During nebulization of the colistin solutions, the interval time was reduced to 15 s because of the much shorter total nebulization times. Measurements were continued until sputtering of the nebulizer or a rapidly decreasing optical concentration.

For the comparative evaluation study, 4 types of nebulizers were tested at 4 different jet pressures with all 4 antibiotics at a suction flow rate of 20 L/min. Relative air humidity and temperature were recorded. A change in performance of the nebulizers was checked regularly with the nebulization of water throughout the study.

Data are presented as volume median diameter (VMD) as derived from the cumulative volume distribution curve as function of the laser diffraction diameter. Unpaired student t-tests were performed for statistical analysis. $P < 0.05$ was considered statistically significant.

RESULTS

Differences in the volume median diameter (VMD) between the different nebulizers for TOBI®.

The mean Volume Median Diameter (VMD) of the TOBI®-aerosol over total nebulization time, obtained at the same constant jet pressure of 1 bar and a suction flow rate of 20 L/min is significantly smaller for LC® Sprint Star (2.88 µm) and Ventstream® (2.36 µm) than for LC® Sprint and LC® Plus (3.75 and 3.65 µm respectively, $p< 0.001$) *(Figure 7.2).* The VMDs produced by Ventstream® and LC® Sprint Star also differ significantly ($p<0.001$). The LC® Sprint is meant to replace the LC® Plus and both types of nebulizers produce the same droplet size distribution indeed. Each bar in *Figure 7.2* is the mean of five complete nebulization runs (vials) until sputtering of the device obtained according to the measuring times and interval times between the measurements as described in the section materials and methods. Hence, the total number of measurements for each inhaler in *Figure 7.2* varied between 39 (for the LC® Plus) and 99 (for the LC® Sprint Start, depending on the time till sputtering and thus, the number of measurements per run. The spread bars in *Figure 7.2* represent max-min for the mean values of the five runs. Relative standard deviations were of the same order of magnitude for all four nebulizers, 6.7; 5.8; 6.6 and 6.2% for LC® Plus, LC® Sprint, LC® Sprint Star and Ventstream® respectively ($n = 5$). For the LC® Plus and LC® Sprint during most of the runs a gradual decrease in VMD was observed (as shown in *Figure 7.3*).

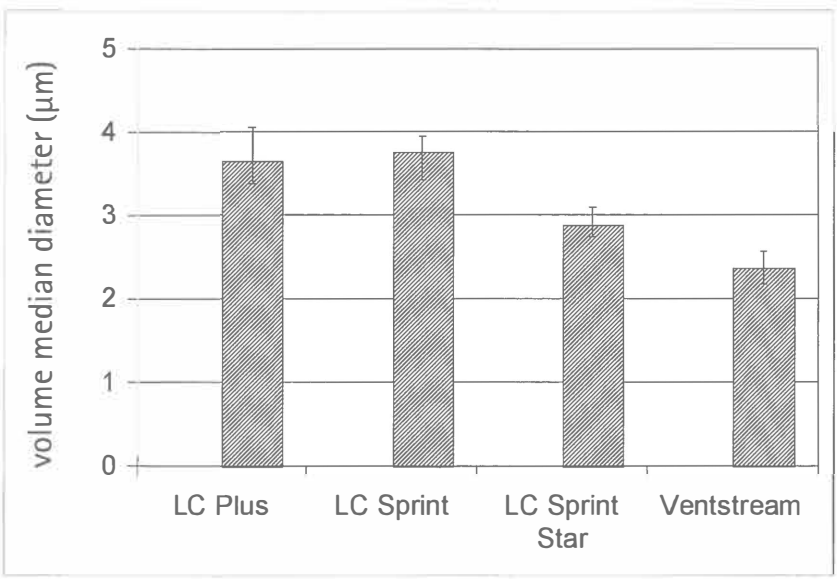

Figure 7.2 *Volume median diameter (VMD) in the aerosol for TOBI® at 1 bar (constant) jet pressure and a suction flow rate of 20 L/min. Mean VMD-values over total nebulization time till sputtering for five TOBI® vials. The spread bars indicate the spread (maximum and minimum values obtained between the five vials). Differences between VMD of LC® Plus/LC® Sprint and LC® Sprint star/Ventstream® P< 0.001)*

For the LC® Sprint Star and Ventstream®, changes in the VMD during the nebulization process were considerably smaller. The most constant size distribution per vial was measured for the Ventstream®. *Figure 7.3* also shows that the variation in size distribution for the LC® Plus becomes larger with increasing nebulization time, i.e. when the amount of drug solution in the nebulization cup becomes less. It is inherent in aerosols from jet nebulizers that the span (X_{90}-X_{10}) increases with increasing volume median diameter. For TOBI® from the nebulizers in this study, the increase in span as function of VMD is linear from 4.77 micron for the Ventstream® to 7.78 µm for the LC® Plus at 1 bar jet pressure (20 L/min suction flow rate). The relative span {(X_{90}-X_{10})/X_{50} for TOBI® from all four nebulizers is almost the same however: 2.06 for LC® Plus; 1.93 for LC® Sprint; 1.97 for LC® Sprint Star and 2.09 for Ventstream®.

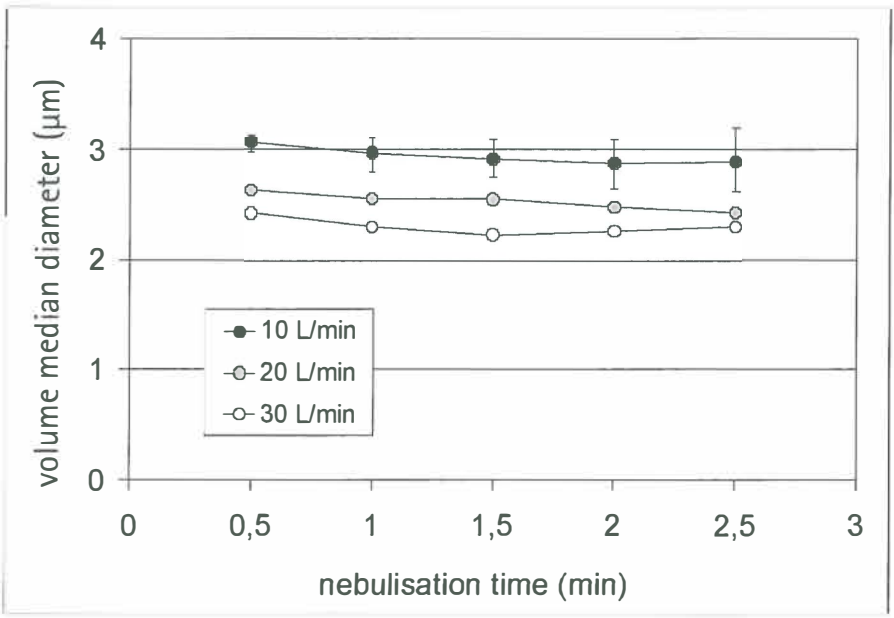

Figure 7.3 *Changes (and spread) in the volume median diameter during the first 2.5 min of nebulization for TOBI® from the LC® Plus at 2 bar jet pressure and suction rates of 10, 20 and 30 L/min. Each curve is the mean of 3 vials. Spread bars in the curve for 10 L/min are indicative for all flow rates and represent the maximum and minimum values obtained at each moment during inhalation.*

The influence of jet pressure and nebulizer on volume median diameter

Figure 7.4 shows the volume median diameter in the aerosol for TOBI® at a suction flow rate of 20 L/min as function of the constant jet pressure. The volume median diameter increases more or less exponentially with decreasing jet pressure. The order of nebulizers from larger to smaller volume median diameter is LC® Plus/LC® Sprint (X_{50} decreasing from 3.50 to 2.20 µm between 1 and 2.5 bar); LC® Sprint Star (X_{50} decreasing from 3.00 to 1.82 µm between 1 and 2.5 bar) and Ventstream® (X_{50} decreasing from 2.50 to 1.70 between 1 and 2.5 bar). On average, the VMDs for the Pari nebulizers (LC® Plus, LC® Sprint and LC® Sprint Star) decrease to the same extent (with 39% between 2.5 and 1 bar). The Ventstream® is slightly

less jet pressure dependent with a decrease of 31% between the same extremes for the jet pressures. With increasing jet pressure, besides a decrease in VMD, also the particle size distribution becomes narrower .

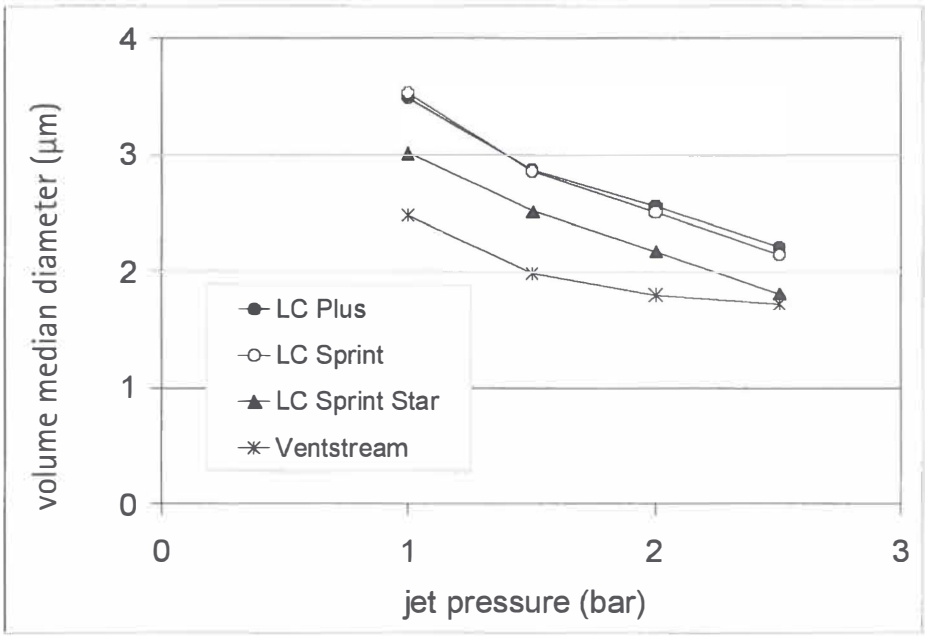

Figure 7.4 *Effect of constant jet pressure on the volume median diameter of TOBI® at a suction rate of 20 L/min for all four nebulizer types.*

Effect of jet pressure source (from mains or compressor) on volume median diameter

Mean jet pressures measured during nebulization of TOBI® at a suction flow rate of 20 L/min for the LC® Plus were 1.74 and 2.67 bar for the TurboBoy N® and CR 60® compressor respectively. Those for the Ventstream® nebulizer for the same compressors were 1.03 and 1.65 bar. Volume median diameters in the aerosols produced by both nebulizers depended on the jet pressure in the same manner as depicted in *Figure 7.4* with higher jet pressure leading to a smaller VMD. Minor differences were found between constant and pulsatile jet pressure but the differences were smaller than the spread (max-min) shown in *Figure 7.2*. As the difference between highest and lowest VMD´s decreases with increasing jet pressure as shown in *Table 7.1*, the source (mains or compressor) for the jet flow results in random but no systematical differences in VMD.

Bar LC® Plus	Bar Ventstream®	LC® Plus		Ventstream®	
		VMD	Max - Min	VMD	Max - Min
1	1	3.65	0.67	2.36	0.39
1.74	1.03	2.54	0.40	2.34	0.23
2.67	1.65	2.19	0.04	1.91	0.19

Table 7.1 *Mean VMD (μm) and absolute spread (difference between minimum and maximum values obtained) in VMD at different jet pressures. VMD and absolute spread for jet pressures (bar) > 1 bar are for all experiments (both constant and pulsatile pressure).*

Effect of suction flow rate on volume median diameter

An increase in suction flow rate from 10 L/min to 30 L/min leads to a slight decrease in VMD for TOBI® from the Pari LC® Plus *(Figure 7.5)*. The effect is independent of the jet pressure with which the aerosol is produced.

The influence of the drug solution on the size distribution in the aerosol

All four drug solutions (2 different antibiotics) appear to yield the same size distribution when they are delivered with the same nebulizer and jet pressure at the same suction flow rate of 20 L/min. Lower rather than higher jet pressures are likely to emphasize differences in aerosol characteristics *(Table 7.1)*. Therefore, the volume median diameters in the aerosols from all four antibiotics and four nebulizers are shown produced at a low jet pressure of 1 bar *(Figure 7.6)*. All differences between the VMD-values from the same nebulizer are within the spread shown in *Figure 7.2*. Therefore, differences have to be considered insignificant and due to the variation in nebulization rather than due to differences in physico-chemical properties of the drug solutions.

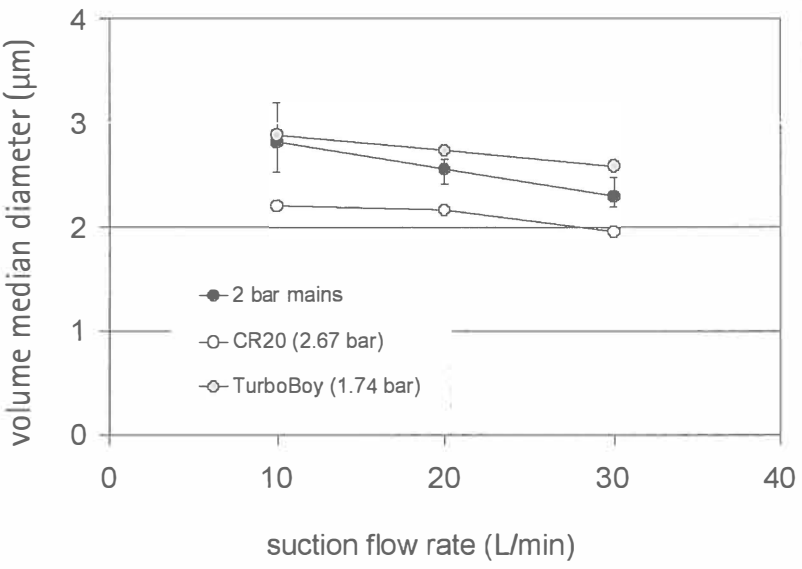

Figure 7.5 *Effect of suction flow rate on the volume median diameter of TOBI® from the LC® Plus nebulizer at 2 bar constant jet pressure (air from the mains) and in combination with the CR60® and TurboBoy N® compressors, yielding 2.67 and 1.74 bar jet pressure respectively.*

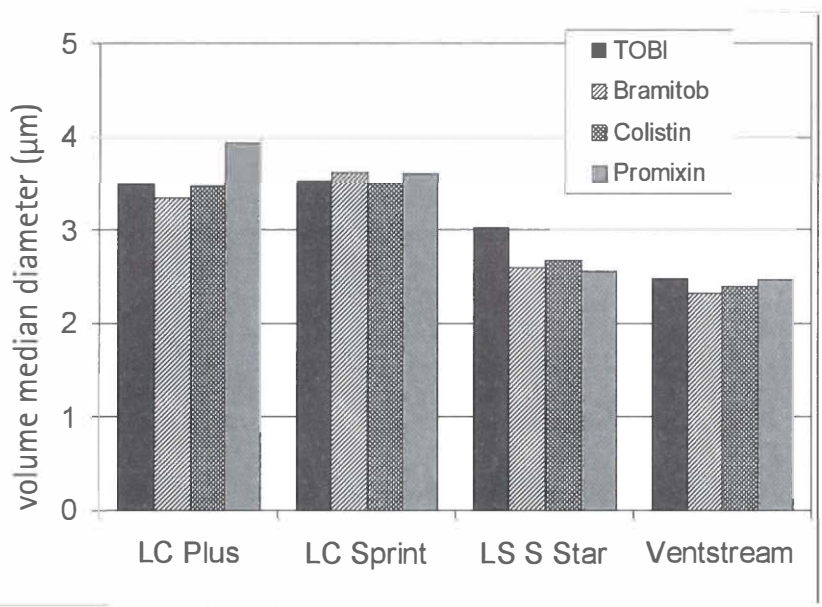

Figure 7.6 *Volume median diameter of all four drug solutions from all four nebulizers operated at 1 bar (constant) jet pressure at a suction flow rate of 20 L/min.*

DISCUSSION

Most importantly this study shows that the VMDs for TOBI®, Bramitob®, Colistin® and Promixin® are the same when the same nebulizer is used at the same jet pressure. Another important finding is that the size distribution (VMD) of the aerosol from the same drug solution may change considerably when a different nebulizer is used at the same jet pressure.

This means that the four drug solutions in this study can be exchanged for used with the same nebulizer without reaching another target area. In contrast, changing the nebulizer for the same drug solution may yield a much finer or coarser aerosol. Considering that antibiotics need to reach the whole lung including the peripheral airways for which small particles in the size range between 2 and 3 micron are more preferable than particles of 3 to 4 micron, the Ventstream® nebulizer seems most suitable for inhaled antibiotics in CF, closely followed by the Pari LC® Sprint Star. Nebulization with the Pari LC® Plus and its successor Pari LC® Sprint results in larger VMDs and must therefore result in lower peripheral antibiotic concentrations compared to both other nebulizers in this study.

Although the VMD from Pari LC® Plus and Pari LC® Sprint (both 3.5 µm) is only 17 to 40% higher than VMD from Pari LC® Sprint Star (2.5-3.0 µm) and Ventstream® (2.5 µm) at 1 bar jet pressure, the difference between the extremes (LC® Plus and Ventstream®) is by a factor 2 expressed in terms of the impaction parameter. This impaction parameter ($IP = \rho.D^2.U$, where ρ is the particle density and U is the particle velocity) predicts the chance of inertial deposition in the upper airways by inertial impaction. The resulting higher value for the impaction parameter may cause a considerably increased impaction of antibiotic aersosols in the upper respiratory tract from, Pari LC® Plus and Pari LC® Sprint with an increased penetration into the central and decreased penetration into the peripheral lung as consequence.

The differences in VMD from the tested nebulizers are likely to result from differences in nebulizer design, especially that for the nozzle and baffle. A difference in design of the nozzle geometry can also be concluded from the difference in jet pressures obtained with the same compressors *(Table 7.1)* for the Pari LC® Plus and Ventstream®. For both compressors across the Ventstream® nozzle a lower pressure drop was found than across the LC® Plus nozzle. This, in spite of a considerably lower VMD from the Ventstream®.

The spread bars in *Figure 7.2* are partly the result of changes in the volume median diameter in the aerosol during the nebulization process *(Figure 7.3)*. Prolonged nebulization (as shown in *Figure 7.3* for the LC® Plus with TOBI®) does not consistently result in smaller particles. Also increased VMDs with increased nebulization times have been observed, e.g. for the LC® Sprint Star. These variations in VMD within total nebulization time do therefore unlikely result from changed physico-chemical properties of the drug solution, as could be caused by evaporation or cooling. The spread tends to increase with increasing nebulization time *(Figure 7.3)* and to decrease with increasing jet pressure *(Table 7.1)*. Further, the differences between maximum and minimum values in *Table 7.1* include all experiments at the same jet pressure. Therefore it was concluded that there is no significant effect of the jet flow source on the droplet size distribution.

As found before, the size distribution in the aerosol decreases with increased jet pressure *(Figure 7.4)*.[18] A higher jet pressure decreases droplet size due to improved break-up of the liquid jet and a shift in the cutpoint of the baffle towards smaller droplets.[19] Almost the same VMD can be obtained from the Pari LC® Plus and LC® Sprint at 2 bar as from the Ventstream® at 1 bar. Further advantages of a higher jet pressure are a higher reproducibility of nebulization *(Table 7.1)* and a much shorter nebulization time.

In contrast with the jet pressure, the effect of the inspiratory (suction) flow rate on the size distribution in the aerosol is relatively small *(Figure 7.5)*. Previously, it was found for TOBI® from the Pari LC® Plus that the effect of suction flow rate decreases with increasing jet pressure.[21] This finding seems to be confirmed for VMD from the CR60® compressor (2.67 bar) compared to VMD from the TurboBoy N® compressor (1.74 bar) and 2 bar from the mains respectively, but not for VMD from 2 bar compared to VMD from the TurboBoy N® compressor. This may be due to the relatively small difference in VMD between 1.74 and 2 bar and the relatively large spread between duplicate measurements at the same jet pressure.

What are the clinical implications of the results of this study? As Psa is present in the whole lung, all airways, central as well as peripheral, should be reached with the antibiotic drugs. In order to do so, the target should be the lower airways as the upper and central airways will receive drug deposition from losses underway. Studies show that this aim can best be reached with particles in the aerodynamic size range of 1 to 3 micron.[19] With the exponentially increasing lung surface area from the trachea to the alveoli, it is clear that the highest possible peripheral deposition has to be the objective in order to obtain the most homogeneous drug distribution in the whole lung. This study provides a tool to the clinician to be informed about VMDs of antibiotics in CF therapy from different nebulizers depending on the jet pressure with which they are operated. This enables the clinician to make a well balanced decision for the type of nebulizer-jet pressure combination to be used by a particular patient. As compressors may decrease in performance over time, measurement of generated jet pressure by a patient's compressor in the outpatient clinic should be tested on a regular basis.[16] This can guide the clinician in estimating which VMD can be expected for the nebulizer used by the patient.

Laser diffraction technique was used to measure the particle size distributions in the aerosols from the nebulizers. The main advantage of laser diffraction technique compared to cascade impactor analysis is the possibility to follow the size distribution during the entire nebulization time by interval measurements. This makes laser diffraction technique appropriate for measuring changes and fluctuations in the size distribution during nebulization. This gives a much better insight in the variations which appear to be the result of both spread within the same measurement and spread between measurements (vials). The fast measurement with laser diffraction technique also enables to complete a large number of experiments, which makes this technique very suitable for comparative evaluation studies.

How do the results of this study relate to existing literature? The Pari company reports an MMD of 3.8 µm for the Pari LC® Plus, 3.5 for the Pari LC® Sprint and 3.1 µm for the Pari LC® Sprint Star, all tested with a 0.9% saline solution using a 1.2 bar compressor and a constant suction flow rate of 20 L/min as measured with Mastersizer X laser diffraction.[20] These MMDs are in good agreement with the VMDs provided in *Figure 7.5* for TOBI® in spite of differences in the properties of the solutions (a low concentrated saline solution compared to higher concentrated tobramicin solutions). Our findings are in contrast with the European consensus of inhaled medication and inhalation devices for patients with CF, in which it is stated that due to a lower surface tension nebulization of colistimethate sodium results in smaller droplets.[13]
An acknowledged limitation of this study is that nebulizer output (rate), which is a result of both nebulization and evaporation, was not formally assessed.

In summary, we demonstrated that the nebulization of TOBI®, Bramitob®, Colistin® and Promoxixin® with the same nebulizer using the same jet pressure results in the same volume median diameter. To obtain an aerosol with the highest fine particle fraction and highest expected total and peripheral lung deposition, Pari LC® Sprint Star and Ventstream® perform best, especially with a jet pressure of 2 bar or higher.

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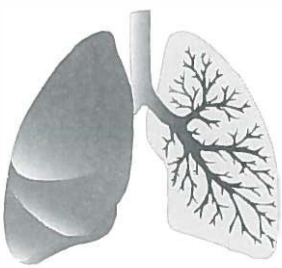
CHAPTER 8



Can improved inhalation technology achieve a successful comeback of inhaled insulin?

Proof of concept

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Abstract

Background

Patients accepted the first insulin dry powder (Exubera®) poorly as the inhaler was large, expensive, available in 2 dose strengths only and complex to use. We aimed to show that improved inhalation technology eliminates these drawbacks whereas systemic insulin availability per mg inhaled insulin can be doubled.

Methods

Fine particle doses (1-3 µm) of Exubera® from the Exubera® inhaler (1 and 3 mg) and a novel disposable dry powder inhaler (Twincer™: 1-12 mg) were measured in vitro using laser diffraction and cascade impactor analysis. Equivalent insulin doses from both devices were subsequently computed and used to treat a 13-year old girl with type 1 diabetes mellitus. Inhalation flow manoeuvres were recorded. Serum insulin levels were measured.

Results

Based on in vitro data, use of the Twincer™ allowed a 42% lower insulin dose compared to the Exubera® inhaler to obtain the same fine particle dose (1-3 µm). Insulin serum concentrations for the 13-year old girl (t = 30 min) per mg inhaled insulin were 7.7 mIU/L when using the Exubera® inhaler and 14.4 mIU/L from the Twincer™.

Conclusions

Systemic availability per mg insulin can be doubled by using the Twincer™ compared to the Exubera® inhaler. With the Twincer™, doses up to 12 mg can be administered in one inhalation. Improved consistency of delivered fine particle dose and hence safety, higher dose flexibility, cost reduction, and patient comfort may fulfil all technical prerequisites for pulmonary delivery of systemically acting drugs, which may facilitate a comeback of inhaled insulin

INTRODUCTION

The International Diabetes Federation estimates that nowadays 285 million people around the world have diabetes.[1] Patients needing short acting insulin could inhale this with a dry powder inhaler instead of taking subcutaneous injections. Despite the potential advantages of inhaled insulin, e.g. no cold chain storage of the drug and disposal of needles, the first insulin inhaler (Exubera®, Pfizer) was withdrawn from the market after just over one year. All other inhaled insulin developments were subsequently terminated, except for the MannKind Technosphere® Technology. Reasons for withdrawal of Exubera® were amongst others poor patient acceptance[2-5], high costs compared to insulin injections[6] and safety concerns.[7,8] The Exubera® inhaler *(Figure 8.1 A)* was a large device and required a large sequence of actions to complete before a dose of insulin could be inhaled. Size and lack of simplicity are drawbacks since devices need to be small and simple for good patient acceptance. Insulin inhalers furthermore should allow a wide range of doses to be taken in one inhalation. For Exubera®, only two doses were available in two different blisters.[8,9] The high costs involved might be reduced by using cheaper inhalers, simple formulation technologies and by increasing the fine particle dose per mg of insulin. The safety concerns with Exubera® involved small lung function changes (for forced expiratory volume in 1 s (FEV_1)) and carbon monoxide diffusing capacity (DLCO) in 1-3 year studies, that all returned to normal after cessation of inhaled insulin treatment.[8] If indeed relevant, changes in lung function may be reduced by increasing deposition efficacy in the target area, which decreases total lung dose. Updated label information by Pfizer and the Food and Drug Administration (FDA) regarding newly diagnosed lung malignancies, all in ex smokers, caused the termination of all competitor developments.[7]

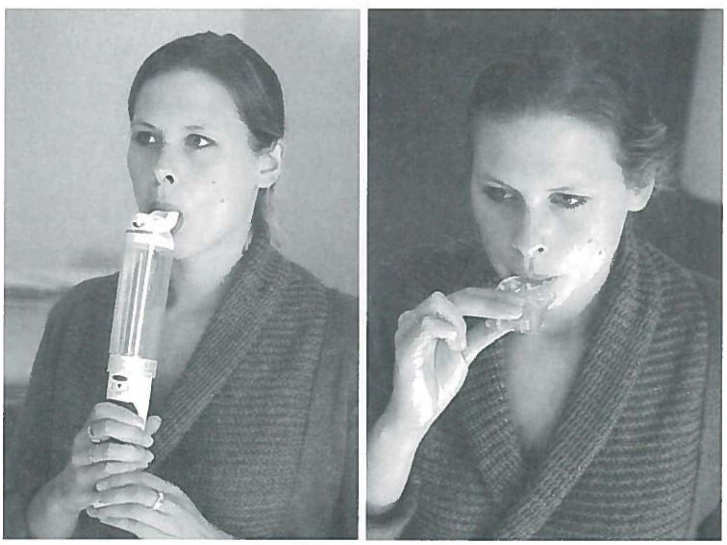

Figure 8.1 *A (left) and B (right) A: The Exubera® inhaler demonstrated by a volunteer B: The Twincer™.*

Do these reasons for termination of Exubera® imply that there is no future for inhaled insulin?[10] The answer depends on the extent to which previously mentioned improvements relative to Exubera® can be realised and the resolution of the safety issues. (Ex)smokers are possibly to be excluded from new trials with inhaled insulin. Clinical studies with MannKind's Technosphere® insulin (Afrezza®) are in a final stage of the FDA approval process. An alternative for inhaled insulin is developed using inkjet technology, but in vitro data from these developments have not been published in the scientific domain so far.[11]

We therefore aimed to show with the Twincer™ dry powder inhaler that improved inhaler technology can fulfil all necessary prerequisites for a comeback, including the same insulin plasma concentrations when giving only half the dose as with the Exubera® inhaler.

MATERIALS AND METHODS

We designed a stepwise approach and determined 1) the delivered doses at different flow rates; 2) the dispersion efficacy and reproducibility of the Exubera® formulation by both inhalers; 3) expected equivalent doses for both inhalers based on equivalent delivered fine particle dose; 4) lung deposition efficacy based on resulting insulin levels.

The Twincer™ *(Figure 8.1B)* is a disposable high dose inhaler developed for the administration of colistimethate sodium in CF patients.[12] The inhaler is also suitable for highly effective pulmonary delivery of a large number of other drugs that need to be administered in high doses. Injection moulded Twincers were supplied by Indes (Netherlands). PVC coated alu-alu blisters of 280 mm^3 (Tommy Nielsen, Denmark) were used as dose compartments. The Twincer™ blisters were filled with insulin powder formulation from 3 mg Exubera® blisters; weighing was on a 5 decimal analytical balance. Exubera® inhalers and insulin blisters (1 + 3 mg) were purchased from EuroCept Pharmaceuticals (Netherlands).

Dispersion efficacy and reproducibility of the Exubera® formulation

Efficacy and consistency of insulin powder dispersion by the Exubera® (1 and 3 mg blister) and the Twincer™ inhaler (1-12 mg doses) were studied with laser diffraction technique, using the primary particle size distribution of the Exubera® formulation from RODOS dispersion (3 bar) as reference. A HELOS BF MAGIC (Sympatec, Germany) laser diffraction apparatus was used with 100 mm lens (measuring range 0.5 to 175 µm). Dispersion testing was at a single flow rate of 55 L/min (2.3 kPa) for the Exubera® low resistance inhaler, and at 35 (2 kPa) and 55 L/min (4 kPa) through the Twincer™ respectively.

Delivered fine particle dose and assessment of equivalent doses

Delivered fine particle doses from the Exubera® inhaler and Twincer™ were analysed with cascade impactor technique (MSLI, apparatus 4 described by the USP/NF 2007). Flow rates were the same as mentioned for laser diffraction analysis and inhalation times were set to 3 s. Fine particle doses from both inhalers within the aerodynamic size range 1-3 µm were computed because this size fraction is considered most appropriate for deposition in the peripheral airways where absorption of insulin must occur.[8,13] From the delivered fine particle doses, equivalent nominal doses from the Exubera® inhaler and Twincer™ were assessed.

In vivo insulin levels

A 13-year old girl was referred to our hospital because of brittle type 1 diabetes (T1D). T1D was diagnosed at the age of 11 years. She was treated with by continuous subcutaneous insulin infusion from the age of 12 years onward. Episodes of hyperglycaemia and ketoacidosis developed gradually with increasing frequency despite an increased insulin dose (5 E/kg/24 hrs). Insulin resistance was considered, but attempts to reduce subcutaneous insulin resistance by adding heparin to the insulin and administration of immunoglobulins were not successful. Next, a trial with Exubera® to improve diabetic regulation was unsuccessful. We hypothesized that improved inhaler technology and the resulting improved alveolar deposition would increase the insulin serum concentrations. The patient was trained to perform a maximal exhalation prior to steady and deep inhalation. Of each inhaler type, one device was instrumented to

record the flow manoeuvres in vivo. The flow curves as function of inhalation time were integrated to obtain the inhaled volumes. After inhalation, a breath hold period (5-10 s) was applied for both devices.

Insulin doses were inhaled at 10 am, noon and 2 pm on 3 successive test days, using both inhalers in an alternating way on top of unchanged subcutaneous pump insulin infusion and a standardized carbohydrate intake. The equivalent nominal dose was based on the glucose level prior to inhalation. Glucose and free insulin levels were determined at t=0, 30, 60 and 90 minutes after inhalation. The samples were treated with polyethylene glycol to precipitate anti-insulin antibodies. The doses administered with the Exubera inhaler were derived from registered dose recommendations for Exubera® in relation to the measured glucose levels[7] those from the Twincer™ were based on the computed in vitro dose equivalence with the Exubera® inhaler. We obtained patient and parental consent for treatment, which was carried out in accordance with the principles of the Declaration of Helsinki as revised in 2000.

RESULTS

We measured that a 1 mg Exubera® blister contains on average 1.8 mg powder versus 5.5 mg in the 3 mg blister. Both the efficacy and the reproducibility of dispersion of the Exubera® insulin formulation are much higher with the Twincer™ than with the Exubera® inhaler *(Figure 8.2)*. This results in a much higher fine particle fraction (FPF) in the aerodynamic size range 1-3 µm with the Twincer™ than with the Exubera® inhaler, being 38.8% and 21.2% of the real dose for a 3 mg blister respectively. For the 1 mg blister, FPF 1-3 µm was 36.7% for the Twincer™ and 22.6% for the Exubera® inhaler *(Table 8.1)*.

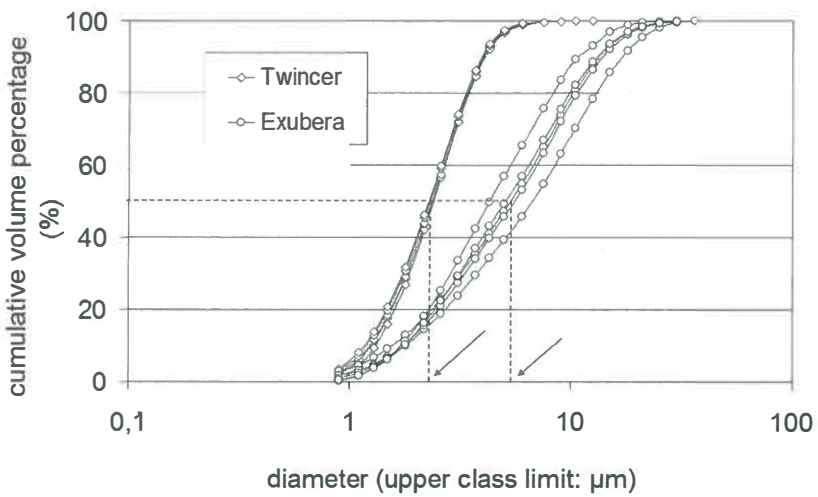

Figure 8.2 *Efficacy and reproducibility of dispersion by the Twincer™ and Exubera® inhaler for five successive (3 mg) doses, both at 55 L/min as expressed with the cumulative volume distribution. The arrows point to the Volume Median Diameter (VMD). The X-axis is logarithmic.*

Inhalor	Dose weight (mg)	MMAD (µm)	FPF <5 µm (%)	FPF 1-3 µm (%)
Exubera®	1*	1.90	32.2	22.6
	3**	2.53	36.3	21.2
Twincer™	2	2.04	52.4	36.7
	4	2.29	57.8	37.3
	6	2.19	60.1	40.3

Table 8.1 *Aerosol characteristics at 55 L/min.*
FPF = fine particle fractions (< 5 µm and 1-3 µm, as percentage of the nominal powder dose (real weight))
MMAD = mass median aerodynamic diameter for FPF < 5 µm from cascade impactor analysis
**1 mg blister contains 1.8 mg formulation; **3 mg blister contains 5.5 mg formulation*

The volume median diameter in the aerosol from laser diffraction for the Twincer™ was 2.7 µm and independent of the dose weight (between 1 and 12 mg) *(Figure 8.3 A)* and the flow rate (35-55 L/min). In contrast with the Exubera® inhaler, although also flow independent, the median diameter was 3.6 µm for the 1 mg blister and 5.4 µm for the 3 mg blister *(Figure 8.3 B)*. The emission time and the volume needed to inhale all insulin form the inhaler for the 3 mg blister at 55 L/min were considerably less for the Twincer™ than for the Exubera inhaler (approx. 1.2 versus 3.7 s for the emission times, equalling 1.05 and 3.4 L of inhaled air respectively). On the basis of the in vitro deposition data in the impactor we calculated that a 1 mg blister (1.8 mg powder) from the Exubera® is equivalent to 1.11 mg powder from the Twincer™ and a 3 mg blister (5.5 mg powder) to 3.02 mg powder from the Twincer™. This is a mean dose reduction by a factor of 1.7 (42%).

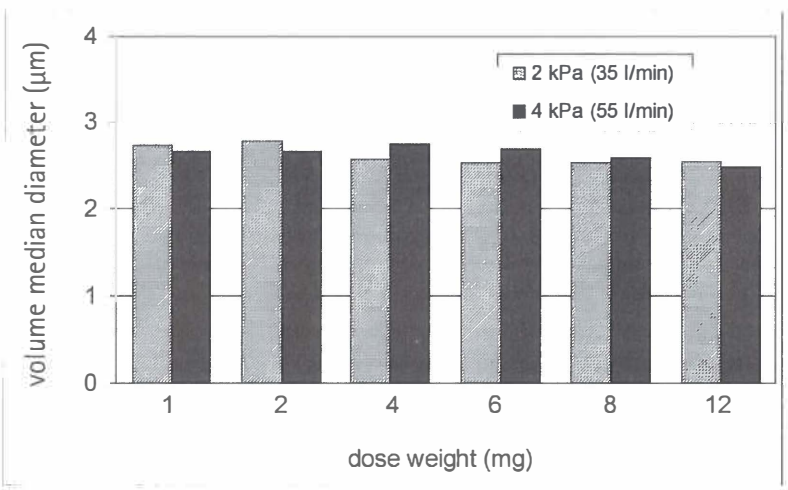

Figure 8.3 A *Volume median diameter in the aerosol from the Twincer™ as function of the Exubera® insulin dose weight at 35 and 55 L/min respectively. Each bar represents the mean of two measurements.*

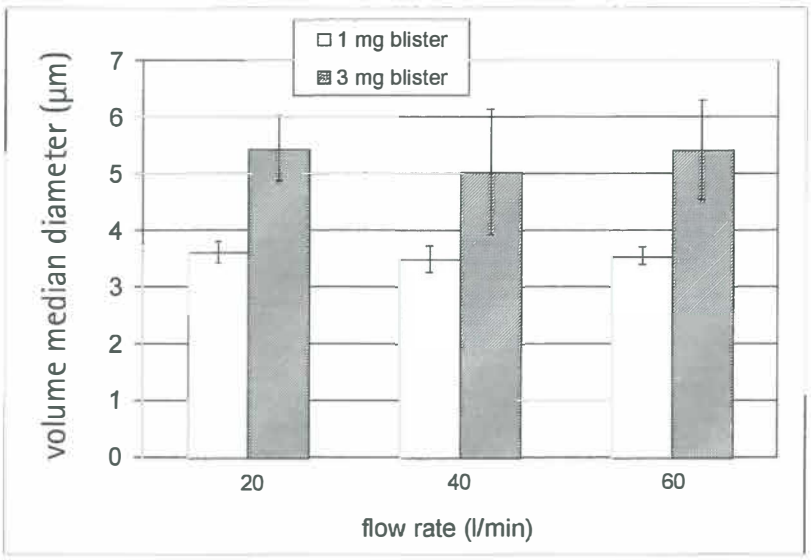

Figure 8.3 B *Volume median diameter in the aerosol from the Exubera® inhaler using 1 and 3 mg blisters at three different inspiratory flow rates. Each bar represents the mean of five blisters.*

With both inhalers, serum insulin concentrations were highest after 30 minutes. The overall mean values for the 3-day treatment demonstrate that the doses needed when using the Twincer™ were 50% lower than when using the Exubera® inhaler. Serum insulin concentrations per mg inhaled insulin were 7.7 from the Exubera® inhaler and 14.4 (mIU/L) from the Twincer™ *(Table 8.2)*.

Inhaler	Mean dose weight administered (mg)	Mean flow rate (L/m)	Mean breathe hold period (s)	Mean insulin plasma concentration (mIU/L) after 30 min	Plasma conc. per mg Exubera® formulation (t=30 min)
Exubera®	14.2	32.9	5.6	110	7.7
Twincer™	7.1	30.7	7.5	103	14.4

Table 8.2 *in vivo serum insulin from the Exubera® inhaler and the Twincer™.*

DISCUSSION

The principle finding of this study is that that the systemic availability of inhaled insulin can be doubled when increasing the fine particle dose by using more effective inhaler technology *(Table 8.2)*. More studies are needed, yet this proof of concept study provides suggestive evidence that inhaled insulin can be a realistic therapeutic alternative for patients with true needle phobia or when there are major needle site problems.

The difference in volume median diameter between the aerosols from the Twincer™ and Exubera® inhaler (e.g. 2.7 µm and 3.6 µm respectively, for the 1 mg blister, *Figure 8.4*) is the main reason for the higher efficacy of the Twincer™. The 33% larger volume median diameter (X_{50}) from the Exubera® equals a 78% higher impaction parameter ($IP = \rho.X_{50}^2.U$), in which ρ is the particle density and U the particle velocity. This impaction parameter is a predictive parameter for inertial deposition in the larger airways. Particles that are only slightly smaller will have substantially higher deposition in the peripheral airways where absorption of insulin must occur.[8,13] The MMAD presented in *Table 8.1* for the 3 mg blister (2.5 µm) is smaller than that from previous in vitro deposition studies with the Exubera system (3.5 µm[14]). Direct comparison of these two MMAD values is not possible however, as we computed the MMAD for the fine particle dose < 5 µm, whereas Harper et al did not specify the fraction for which they computed the MMAD.

The even slightly greater improvement in systemic availability (2x) compared to the in vitro deposition efficacy (1.7x) may be explained by a much shorter emission time from the Twincer™ than from the Exubera® inhaler. For effective systemic delivery via alveolar absorption the entire dose should preferably be delivered within the first litre of inhaled air to transport the aerosol to the peripheral airways. If the aerosol is diluted in a larger volume (as for the Exubera® inhaler), part of the dose may not reach the alveolar absorption area and total residence time in the lungs may be too short for effective sedimentation. A breath hold of 5-10 seconds following inhalation further improves deep lung deposition.

Our results suggest that inhaled insulin can be a realistic alternative for patients with T1D needing a pre-meal bolus of insulin. Pharmacokinetic characteristics of inhaled insulin with rapid absorption and rapid elimination compared to subcutaneously administered insulin closely mimics endogenous prandial insulin release.[15] The higher in vitro fine particle dose from the Twincer™ in the 1-3 µm range compared to the Exubera inhaler corresponds well with the increased systemic availability of insulin in vivo. This particle fraction of 1-3 µm is regarded optimal for peripheral airways deposition.[13] A lower insulin dose with a cheap inhaler device might significantly reduce costs as well. Other advantages are that the Twincer™ is small, disposable and easy to use. Dose flexibility can be increased to 12 mg without affecting dispersion efficacy. Regarding safety, the conclusions for different products (Exubera®, AERx®, AIR®, Afrezza®) after 1 to 3 year use of these systems are that lung function changes are relatively small, non-progressive and reversible. Reported lung function changes may also be attributed to T1DM being a micro angiopathic disease also affecting the lung.[16] Although a causal relation between inhaled insulin and the development of lungcancer has not been demonstrated, an increased concentration and duration of exposure of the alveolar epithelium and underlying connective tissue to insulin should possibly be avoided.[15,17] With a more homogeneous distribution with a higher small particle fraction, local insulin concentrations may be lower and the transfer time from alveolus into the circulation may be shorter. We hypothesize furthermore that he reported lung function changes may partly have been caused by the excipients used in the insulin formulations. Exubera® contained mannitol, glycine, sodium citrate and sodium hydroxide. The Technosphere® formulation (Afrezza®), the only remaining system in development, consists of highly porous fumaryl diketopiperazine self-assembling crystals with a large surface area to which insulin is absorbed. Regarding long term safety, excipient-free insulin might be preferable.

The importance of this study is that we have shown that improved efficacy and consistency of lung deposition, increased dose flexibility, cost reduction and increased patient comfort are all feasible with improved inhaler technology. This concept was applied in one girl with T1D with success. Future studies in larger groups of patients have to show the implication for implementation in the management of T1D including long term safety issues. This may fulfil all technical prerequisites for pulmonary delivery of systemically acting drugs and a comeback of inhaled insulin for selected patients.

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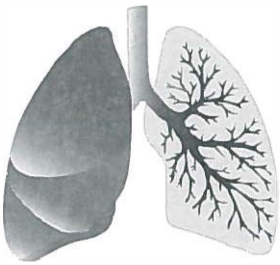
CHAPTER 9



General discussion

Including:
Aerosol drug delivery:
developments in device design and clinical use

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GENERAL DISCUSSION

9.1 Inhaled drug delivery: many variables and their interactions

The ultimate aim of this thesis is to improve treatment of (respiratory) diseases by improving inhalation treatment. Inhalation of aerosolized drugs has resulted in better clinical control of prevalent pulmonary diseases like asthma and COPD. Inhalation therapy has also led to an important increase in life expectancy and quality of life for patients with Cystic Fibrosis. Further, the pulmonary route has proven to be suitable for systemic drug delivery, e.g. for insulin and vaccines. A high fine particle fraction is necessary for optimal targeting of the peripheral airways for respiratory diseases as well as for systemic treatment and the delivery of vaccines by inhalation. However, despite clear advantages of inhaled therapy and ongoing device and drug formulation development, incomplete understanding of the many factors that influence drug deposition in the respiratory tract has been a limiting factor in achieving optimal inhalation therapy so far.

The results of our studies clearly demonstrate that relevant improvements are both possible and necessary. In this thesis, we have provided insights in factors contributing to lung deposition. First, we showed that the choice of drug and device combination significantly influences the (fine) particle output from a pMDI. The output of fluticasone 250 and 125 µg/dose pMDIs decreases with the number of delivered doses *(chapter 3)*.[1] Fine particle dose when pMDIs are used with valved holding chambers (VHCs or spacers), as recommended in children, may vary from 17% to 37% of label claim only depending on which pMDI-spacer combination is chosen *(chapter 4)*. Second, increasing air humidity leads to up to a 50% higher output from VHCs without a change in particle size distribution and thus results in a higher fine particle dose *(chapter 4)*. For nebulized drugs, a relevant increase in fine particle output can be obtained by a higher jet flow through the nebulizer system *(chapter 7)*. Third, systemic absorption of insulin can be doubled by using an appropriate device which enables adequate inhalation and delivers a higher fine particle fraction in the initial phase of inspiration compared to an earlier insulin inhaler *(chapter 8)*. Treatments are time consuming. The results in *chapter 6* additionally show that lack of adherence to cleaning regimes may lead to a large and clinically relevant decrease in output from nebulizer systems.[2] Finally, we show that two marketed tobramycin solutions with different drug concentrations and different excipients do not differ significantly in aerosol properties when nebulized with different nebulizer-compressor systems. Therefore, these products are widely exchangeable when used with the same system. For 2 different suspensions of colistin, results were similar *(chapter 7)*.

The studies above give insight in some characteristics relevant to the choice of aerosol generation device, environmental circumstances and drugs. However, reaching the exact target area in the lung for drug deposition with a sufficient fine particle dose requires more than the appropriate selection of the drug and the device needed. Many variables are involved in inhalation therapy and many interactions between these variables exist *(Figure 9.1)*.

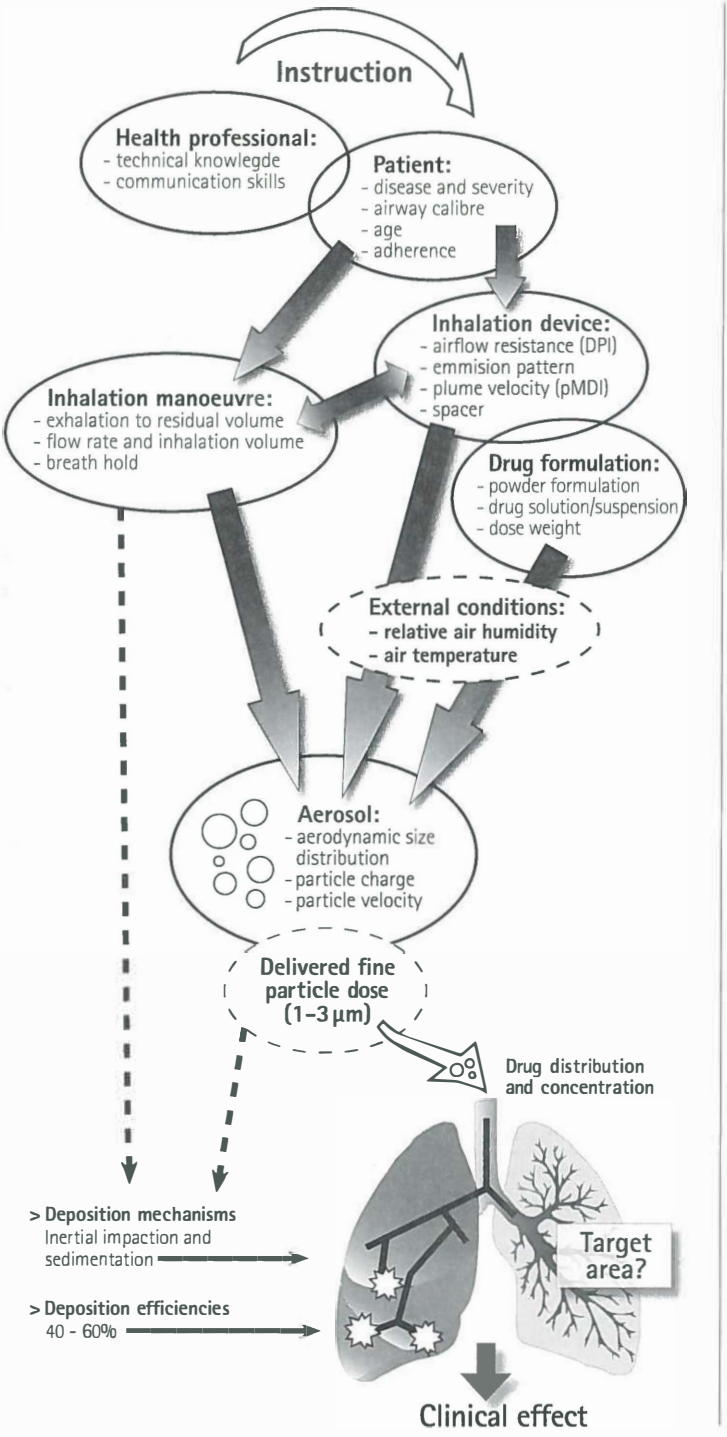


Figure 9.1 *interaction of the many variables in drug delivery in the airways.*

The health care provider has to select the drug and an appropriate device for inhalation, depending on the type of disease, disease severity, age and skills of the patient. The health care provider then has to instruct the patient about the best procedures for the chosen inhalation device and the patient has to be motivated to comply with these instructions. For nebulizers and spacers this also includes the need of cleaning. In *chapter 1* basic principles of inhalation therapy are discussed.

Inhalation manoeuvre

Exhalation to residual volume before inhalation and a breathhold pause (theoretically as long as possible, in practice 5 to 10 seconds) after maximal inhalation may result in a peripheral lung deposition of 25%. This sequence is essential for optimal use of dry powder inhalers and also recommended for children using a pMDI-spacer combination whenever possible.[3,4] Exhalation to residual volume results in maximal air refreshment of the most distal airways with inhalation. Slow to moderate inhalation flowrates decrease impaction in the upper airways. The breathhold results in more time for sedimentation to occur. This manoeuvre is optimal for drug delivery in the small airways and in the alveolar region.

Inhalation device

The intrinsic inhaler resistance of a DPI affects the peak flow rate and duration of inhalation. Over a low resistance inhaler, the instruction to inhale forcefully will lead to increased drug losses in the oropharyngeal region by inertial impaction. Only if the DPI compensates this with a higher fine particle dose at higher flowrates, the distribution of deposition in the smallest airways may remain similar. For pMDIs, drug losses in the oropharynx or spacer may increase by a high aerosol plume velocity resulting in increased impaction. For deep lung penetration of the drug, the aerosol emission time from a DPI or nebulizer has to be short and early during inhalation as only the fraction of the dose delivered within the first half of the inhaled volume will be effectively transported into the alveoli and most distal airways.

Drug formulation

The drug formulation itself can have a great effect on the properties of the aerosol and the dose delivered to the patient. From adhesive mixture types of powder formulations, generally less than 50% of the dose is released as particles with the appropriate aerodynamic size distribution. Surface tension and viscosity influence the size distribution of the droplets from nebulizers and may change during the nebulization process due to evaporation and temperature changes. Relative air humidity may also have an effect on hygroscopic powder formulations in DPIs, especially when stored under moist conditions over longer periods.

In conclusion, many human-drug-device interactions exist and these may all result in increased or decreased deposition in the target lung area, subsequently leading to increased or decreased symptom- or disease control. These interactions will be addressed in the next parts of this chapter, focusing on target area, fine particle dose and drug distribution, patient related factors and methods to measure effectiveness of inhalation therapy.

9.2 Target area

To improve drug delivery to the lungs in pulmonary or systemic diseases, first the target area needs to be defined and 3 issues need to be addressed.

To start, it is necessary to define the target area to which the drugs need to be delivered. This target area depends on the location of the disease process (e.g. the site of inflammation or infection), the presence of drug receptors (e.g. steroid receptors) and effector tissue (e.g. smooth muscle).

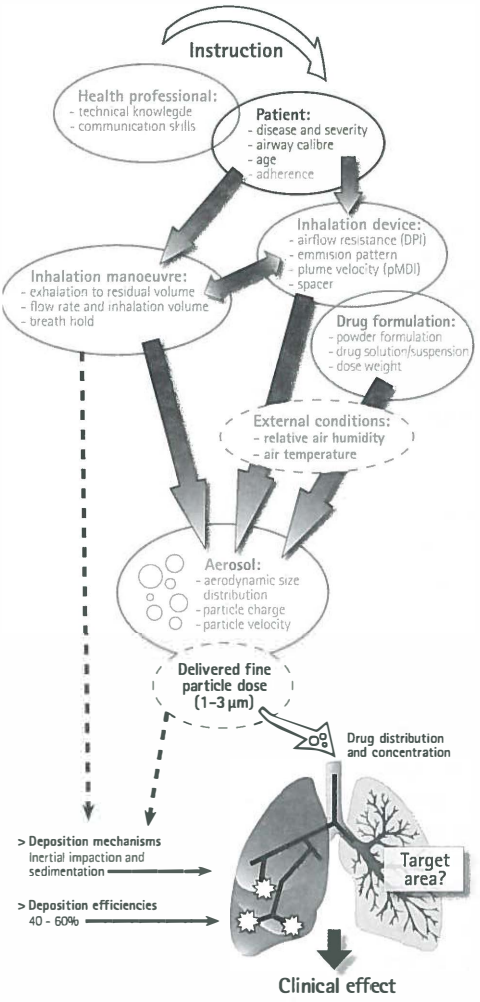
Second, the chosen target area is the principal determinant of the preferred aerodynamic particle size (distribution) and optimal inspiratory flow manoeuvre .

Third, devices need to be developed and selected that facilitate patients from a wide age range to use the device correctly, resulting in optimal output and deposition at the most favourable inspiratory manoeuvre.

Asthma and COPD

Asthmatic inflammation is present in both large and small airways[5,6] with an increasing number of ICS receptors towards the periphery.[7] In asthma, all airways thus need to be targeted with ICS with emphasis on the peripheral airways. The target area for beta-2-agonists in asthma is predominantly determined by the airway generations where smooth muscle contraction leads to airway narrowing. Although smooth muscle is present all the way down to the respiratory bronchioli, in airways distal to generation 14, other stretching and pulling factors play a role in keeping the airway opened. Down to generation 7, airways are to some extent protected from narrowing due to smooth muscle contraction by cartilage. Therefore, the target area for bronchodilators are the airways from generation 7 to14.[8]

Chronic obstructive pulmonary disease (COPD) is an inflammatory disease where the whole



respiratory tract is affected from the central to the so-called small airways of less than 2 mm in internal diameter.[9] Depending on severity as expressed in GOLD stage 1-4, treatment varies from short acting bronchodilators as needed to long-acting bronchodilators with or without ICS (www.goldcopd.org). The target area for both ICS and long acting bronchodilators in COPD is therefore similar to that in asthma.

CF

In CF lung disease, infection (e.g. by *Pseudomonas aeruginosa, aspergillus fumigatus*), viscous sputum and inflammation play an important role and may be targeted by inhaled antibiotics or antifungals, DNAse and hypertonic saline, and ICS respectively. Small airways in CF are involved in the disease process from birth onward, as indicated by trapped air on CT scans[10] and by increased FRC as measured with pulmonary function tests in young children, which reflect inflammatory processes and mucus plugging.[11,12]

Pseudomonas aeruginosa (PA), if present, resides in both conductive and respiratory airways.[13] Therefore the target area for nebulized antibiotics in CF are all airways and a high fine particle fraction is needed to target these all. For the treatment of PA in the airways with aminoglycosides this is even more important as the efficacy of aminoglycosides is predicted best by the peak concentration (C_{max}) to Minimal Inhibitory Concentration (MIC) ratio and a C_{max}/MIC ratio greater than 10 is generally advised. For tobramycin this means that a concentration of 20 mg/L in the airway may be necessary, underscoring the need for targeting the small airways to reach this high concentration in the light of an exponentially increasing airway surface area *(chapter 5)*.[14]

Viscous mucus is present in all airways in CF and airway obstruction starts, in young children already, in the small airways. Mucolytics therefore need to target the entire bronchial tree, and in particular the small airways.

In summary, all airways may need to be targeted with anti inflammatory, antimicrobial and mucolytic drugs in CF.

Systemic delivery

The alveolar area is the target area for systemic absorption. Drug losses in conductive and transitional airways should be kept to a minimum, as medication landing here is less likely to reach the vascular endothelium where absorption in the systemic circulation can take place.

How to achieve the highest drug concentration in the target area.

To achieve optimal clinical effect, most of the drug dose should be deposited in the target area for that drug. Whether this is possible or not, depends on where the target area is located. When the upper and central airways are to be treated (as with bronchodilators), larger particles can be used theoretically and the velocity with which these particles are released into the respiratory tract can be higher than for peripheral lung deposition. The particle momentum (the product of mass and velocity) should not be too high however, to prevent substantial losses in the oropharynx, which reduces the dose available for the lung. Because of the small surface area of the upper and central airways, high drug concentrations can be achieved in these areas. However, the surface area of the airways increases exponentially towards the alveoli. Due to this increase and the drug losses in the upper airways *(chapters 1 and 5)* it is impossible to reach the same high concentrations in the peripheral lung. Neither can a homogeneous drug distribution within the whole lung be achieved: regardless of particle size, there will be a decrease in drug concentration from large to small airways because of this exponentially increasing surface area and the approximate 1:1:1 deposition ratio in the conducting, transitional and peripheral airways.[15-17] In a study with monodisperse particles of salbutamol of 6, 3 and 1.5 µm, particles were inhaled with a relatively slow inhalation of 30 L/min followed by a breathhold period. This resulted in an increase in peripheral lung deposition from 10 to 25% with decreasing particle size from 6 to 1.5 µm.[18] 25% of the total dose of

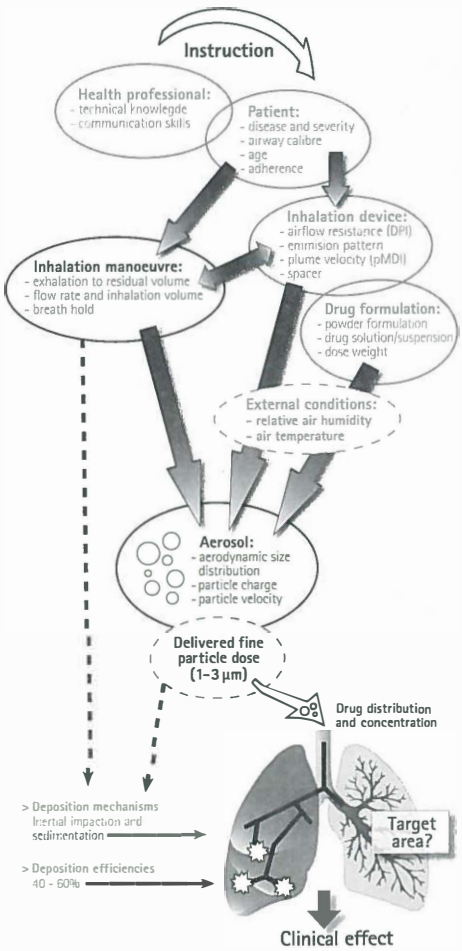
1.5 µm particles deposited on an estimated 95% of the lung surface area whereas 31% deposited on the other 5% of the total lung surface area resulting in a almost 25 fold mean concentration difference. For larger particles, this concentration difference is even larger. Using an inhalation flow of 67 L/min resulted in a lower peripheral lung deposition for all three particle sizes compared to the slower inhalation flow.

Therefore, when the small airways are the target area, drug concentration there will inevitably be lower than in higher airway generations.

9.3 Particle size and inhalation manoeuvre: it takes two to tango

The interaction between particle size and inspiratory flow rate determines deposition. Ideally, ICS particles should be 1.5 µm for optimal small airway targeting and should be inhaled with a low to moderate flow rate; currently, a range of 1-3 µm is the most feasible size fraction from all types of pMDIs, DPIs and nebulizers. For bronchodilators the particle size fraction can be broader (3-5 µm) as the target area is confined to airway generations 7-14. Indeed, it has been shown that for bronchodilators larger particles are more efficacious and achieve more bronchodilation than smaller particles with a change in FEV_1 of 551 ml for 6 µm particles, 457 for 3 µm and 347 for 1.5 µm.[18]

With a slower and longer inspiration, the same area can be reached with a slightly larger particle size as with a faster and therefore shorter inhalation of slightly smaller particles. This has been confirmed in quite a few studies. As an example, in a radionuclide study comparing nebulizer generated aerosols with an MMAD of 5 µm with a 9.5 µm MMAD aerosol, an extremely slow inhalation of less than 0.5 L/min during 10 seconds resulted in a higher conductive airway deposition of the large particles compared to cyclic tidal breathing of the smaller particles.[19]



Targeted delivery to smaller airways with saline solution droplets (1 and 3.7 µm droplets) mixed with the radio isotope (99m)Tc was possible with smaller droplets inhaled at a lower (18 L/min and 38 L/min) inspiratory flow rate from functional residual capacity.[20] Other studies also show that regional lung deposition is dependent on particle size, inhalation volume and flow rate.[21] Nebulization of tobramycin inhalation solution with the eFlow® rapid reduced nebulization time, but also resulted in a 40% reduced whole lung deposition compared to LC® plus nebulizers.[22] This is in agreement with our study presented in this thesis, in which the particle size distribution of the eFlow® rapid is shifted to larger particles compared to Pari LC® plus *(chapter 6)*.[2] Of interest, in healthy subjects, lung deposition of both devices was comparable. The larger particles may impact easier in more severely obstructed airways. A high fine particle fraction is necessary to reach beyond diseased airways with reduced airway caliber.[23]

For younger children using a pMDI-spacer combination with a face mask, quiet breathing is essential. Crying has proven to reduce lung delivery in nebulized budesonide due to the high flow rates generated and the resulting impaction in upper airways.[24]

Although a large fine particle dose should be more effective than an equivalent dose of coarse particles, this has not been conformed in vivo so far. Reasons for that may be that many studies are of too short duration, compare different drugs and devices in the same study, and do not report on inhalation parameters and environmental conditions that are all important as we have shown in this thesis. Further, the exponentially increasing surface area of the lung towards the periphery can result in too small differences in deposition and therefore in clinical effectiveness. To investigate this, the power of studies should be increased by enrolling more patients and/or studying them for a longer duration. Finally, the plateau of the dose-response curve may have been reached, so that a higher local drug deposition, e.g. in more peripheral airway targeting, does not result in a clinical difference.[25]

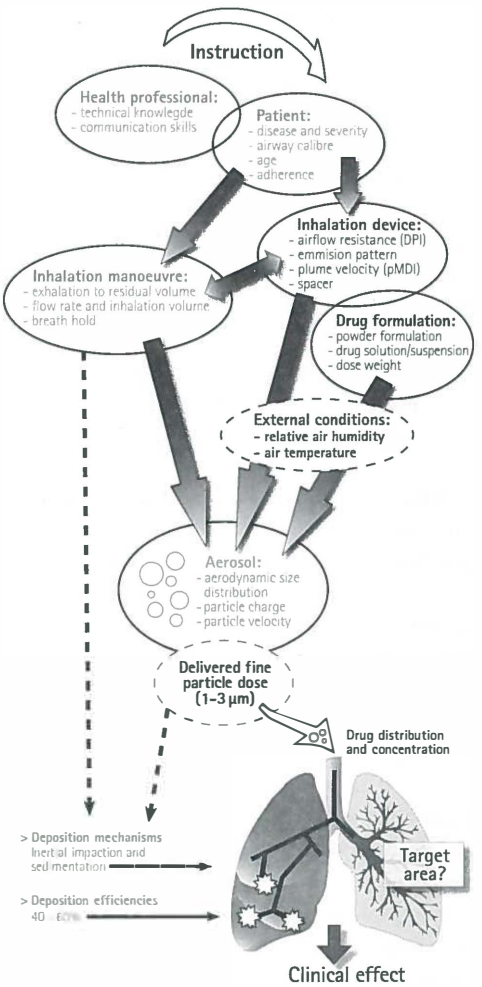
9.4 Fine particle fraction: devices and conditions

The absolute fine particle fraction from a drug dose primarily depends on the choice of delivery device. In this thesis we demonstrated that this is true for pMDI's, pMDI-spacer combinations, DPI's and nebulizer systems *(chapters 4-8)*.
Although this finding is not new, the extent to which differences between delivered doses exist, surprisingly high: they are up to a double dose and can thus be considered clinically relevant.

As expected, pMDI's differ in their delivered fine particle fraction (1-3 µm volume median diameter (VMD), however the differences in delivered fine particle dose are unexpectedly high between 38% of label claim for fluticasone (125 µg/dose) and 61% of label claim for ciclesonide (160 µg/dose)*(chapter 4)*. These large differences result from the formulation. Valved holding chambers or spacers reduce the fine particle dose released from the spacer (and thus the dose available for inhalation), leading to a further reduction of the fine particle dose.

For fluticasone with Volumatic® this further reduction is 44% of the 38% fine particle dose directly from the pMDI and 60% of 61% for ciclesonide with the (non anti static) AeroChamber®. These reductions of fine particle dose due to losses in the spacer are considerably less at higher humidity *(chapter 4)*. We hypothesise that losses in the spacer are less with increased humidity, primarily because of reduced electrostatic charge; electrostatic charge that continues to play a role despite washing (or priming) of the spacers.

For pMDIs and valved holding chambers we need to know the output of the various drug-device combinations as function of the environmental conditions (e.g. air humidity). For pMDI-spacer combinations, ICS fine particle output increases with higher humidity. Ciclesonide and HFA-BDP



both had highest fine particle output with the AeroChamber Plus® *(chapter 4)*.

For dry powder inhalers (DPIs) the output depends on effective drug dispersion, which is often dependent on the pressure drop across the inhaler. A high pressure drop is preferred as it results in a lower inspiratory flow rate compared to a low pressure drop. Further, a flow dependent increase in fine particle dose release can compensate for impaction in upper airways associated with

increased flow rates.[26] Release of the dose early during inhalation enables relatively peripheral deposition *(chapter 8)*.

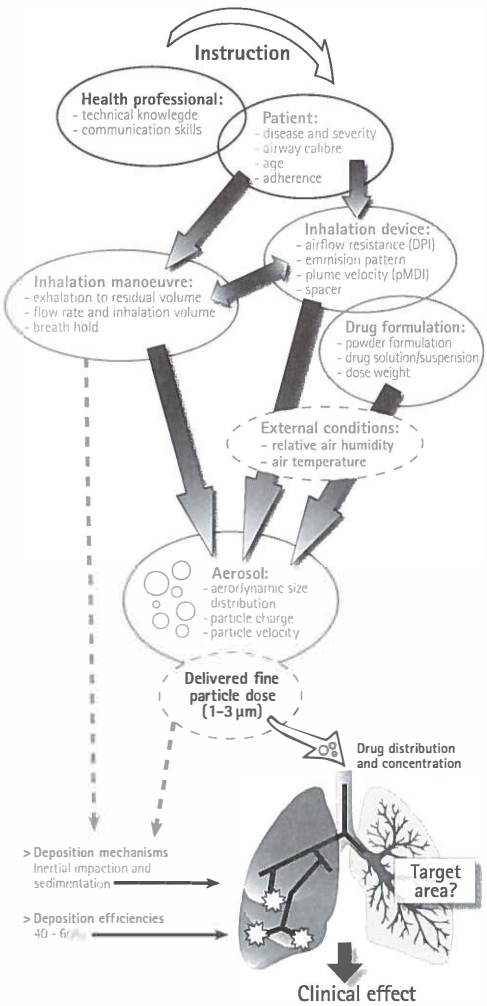
Nebulizer-compressor systems can be used with different drug solutions and suspensions, but the properties of the aerosol and output rate should be investigated for each drug formulation. Moreover, once the aerosol properties for a particular drug formulation from a nebulizer are known, no changes in type of compressor or jet flow can be made without changing the aerosol properties too. For nebulizer-compressor systems, fine particle output is dependant on the choice of a nebulizer and it increases with increasing jet-flow *(chapter 7)*.

9.5 Patient related factors: inhalation instruction and adherence

As lung deposition of inhaled drugs generally increases with age, children and adults can use the same nominal dose of inhaled drugs. A few studies provide some evidence for this statement, e.g. a pharmacokinetic study with budesonide[27] and a study with Technetium labeled salbutamol. For young infants with the nose as an effective filter the dose needed might be higher.[28]

Inhalation instruction is crucial in inhalation therapy and should be repeated frequently to ensure a correct inhaler technique.[29] Probably the most important determinant of the success of inhalation therapy is adherence. In clinical trials in pediatric asthma, adherence to treatment as monitored by electronic devices may be as low as 50%-77%.[30] As participation in a trial usually increases adherence, these percentages are presumably (much) lower in a non-selected asthmatic population.[31]

Also, adherence to cleaning instructions is essential. In a study with a mesh nebulizer, inadequate cleaning resulted in loss of drug output. The mesh needs to be replaced timely as the volume median diameter increases over the course of time *(chapter 6)*.[2] Increasing of adherence to treatment and cleansing may therefore be considered an effective and cheap



way ("low hanging fruit") to improve disease- and symptom control and high priority should be given to research related to improving adherence in asthma and CF patients.

9.6 How to measure pulmonary drug delivery

This thesis provides exact data on the total output and the fine particle fraction from 4 ICS delivered by pMDIs without and with 4 valved holding chambers and on 4 antibiotics delivered by 5 different nebulizer systems under various conditions. Also 2 dry powder inhalers for insulin were compared with each other *(chapters 4-8)*. We have shown that an optimal fine particle fraction may be obtained by selecting the best devices and optimizing environmental factors as air humidity. Now that an optimal fine particle fraction can be generated, this leads to the most important question how these findings relate to daily life, or in other words, how do aerosol doses delivered at a patient's mouth relate to pulmonary deposition and clinical effects? Ideally, the most relevant outcome of drug delivery to the lungs would be the clinical effect (no symptoms, no exacerbations). However a direct relation between drug dose and clinical effect may be difficult to measure, as the clinical response may be influenced by many other factors than the location of deposition only.

Which factors affect the clinical response to medication in asthma? For children with asthma, asthma severity, duration of asthma (maybe related to airway remodeling), baseline pulmonary function and pharmacogenetic make-up can obscure the dose-response relation and influence clinical effect. Both response to bronchodilators, as response to ICS are subject to these factors.[32-36] In preschool children outcomes are even more difficult to define, in particular as pulmonary function tests like flow-volume loops cannot be performed.

For patients with cystic fibrosis, factors that obscure a direct dose response effect are the different mutation classes in CF, the presence of modifier genes and disease progression.

Therefore other methods of determining lung deposition are used as a proxy for clinical effectiveness and will be explained for asthma and CF in the next paragraphs.

Other methods of determining lung deposition as a proxy for clinical effectiveness in asthma

Information on the area of deposition for ICS can be obtained directly from radio-labeled studies of aerosols with gamma scintigraphy or PET-scanning.[37] In general, pulmonary deposition measured with gamma scintigraphy can be expected to be slightly elevated as it measures also a proportion of drug that may subsequently be removed from the lungs by mucociliary clearing mechanisms.[38]

Information on deposition can also be obtained indirectly from plasma sampling after an inhaled dose[32,39], urinary excretion, pulmonary function tests like peripheral airway resistance, measurement of lung clearance index and changes in ventilation heterogeneity[40,41], air-trapping on expiratory CT scans[42,43], gas distribution as measured with the use of hyperpolarized helium with magnetic resonance imaging (MRI)[44,45], from side-effects like endogenous cortisol suppression[46] and lung models.[47,48]

Deposition based on clinical effectiveness

For bronchodilators, information on the deposition has been obtained by measuring effectiveness as change in FEV_1 and measuring side effects by measuring heart rate and tremor. A study measuring the speed of bronchodilator response to salbutamol showed that the relative potency of pMDI and Volumatic® was twice that of the Diskus®. The results also indicate that it would require approximately twice the dose of salbutamol administered via the Diskus to obtain the same recovery times as those recorded when salbutamol is administered via the pMDI + Volumatic®.[49] In general, studies on clinical effects do not report on humidity conditions with spacer use, which, as we have shown, are important *(chapter 4)*, nor on inspiratory manoeuvre and whether a breathold was applied with the DPI *(chapter 8)*.
Lung deposition may be assessed by measuring side effects of inhaled medication. To give an example, the lung bioavailability of fluticasone propionate as expressed by the level of adrenal suppression was about twofold greater with hydrochlorofluorocarbons than with hydrofluoroalkane as propellant, and therefore these 2 pMDIs are not interchangeable.[50]

Symptom free days and nights, time to first exacerbation, emergency visits and hospital admissions are common endpoints in randomized controlled trials on the clinical efficacy of ICS. To give an example, for ICS, the dose equivalence of CFC-BDP to HFA-BDP has been established as a 2.6:1 ratio based on an efficacy study in adults. In this RCT, 323 adults with asthma received either 100, 400 or 800 µg of CFC or HFA-BDP over 6 weeks and based on the shift of the FEV_1 dose-response curve to the left for CFC-BDP the 2.6:1 ratio was calculated.[51] In a dose reduction study, budesonide by Turbuhaler® and fluticasone by Diskhaler®, both drugs and devices were equally effective in a µg for µg comparison.[52]

Increased ventilation heterogeneity in the small airways, as can be measured with the concentration of a tracer gas with the multiple breath wash-out technique, is a sensitive marker of small airways disease and associated with poor asthma control.[41] From this multiple breath wash-out technique, parameters can be derived that reflect ventilation heterogeneity in convection-dependent airways [S_{cond}] and ventilation heterogeneity in diffusion-dependent, acinar airways [S_{acin}]. A low S_{acin} reflects increased involvement of peripheral airways in patients with asthma, and it was only the patients with increased involvement of peripheral airways that had benefit from switching from equivalent doses by budesonide DPI to HFA-BDP.[40] Of note, all patients with asthma also presented conductive airway abnormality at baseline, but no changes were observed in this lung zone with the switch to the ultrafine aerosol HFA-BDP.

Deposition based on pharmacokinetic studies

With pharmacokinetics, blood levels of an inhaled drug are measured after the drug has been absorbed from the lung. Usually the plasma concentrations are plotted in an area under the curve graph and compared to the systemic availability of the same drug given intravenously. A caveat in pharmacokinetics from inhaled drugs is that these drugs may enter the systemic circulation not only by the pulmonary, but also via the gastro-intestinal route. This gastrointestinal route may be blocked by charcoal ingestion. The systemic bioavailability may also be influenced by first pass metabolism by the liver. For example, this first pass metabolism is responsible for the very low oral bioavailabilty of fluticasone being less than 1%.[53] Salbutamol pharmacokinetics showed large variation depending on anti-electrostatic handling of the plastic spacer in pediatrics, with primed or used spacers delivering an up to two fold higher dose than spacers that had not been used before, or primed but just rinsed with water.[54] Another study on salbutamol inhaled with Diskus® (DPI), Diskhaler® (DPI) or Easy-Breathe® (breath actuated device) found that in healthy volunteers, the Diskus® resulted in significantly lower bioavailability as a surrogate for

lung delivery.[55] Lung deposition and systemic availability of budesonide with three different nebulizer systems were all 14%-16% and 15-17% respectively of nominal dose in adults, When related to dose-to-subject or actual dose, despite the greater drug output from the nebulizer because of the lowest residue in the reservoir, lung delivery and systemic availability for this nebulizer producing 7 µm (MMAD) particles was relatively low with 36% and 44% respectively compared to those values for the nebulizers with 5 (58% and 63%) en 3 µm particles ((59% and 64%).[56] Ciclesonide inhaled directly from pMDI or from AeroChamber Plus® resulted in equal serum concentrations in adults[57]; currently ciclesonide is not registered with a spacer.

Deposition based on CT and MRI imaging studies

Another surrogate for lung deposition might be endpoints on CT or MRI scans. HFA-BDP treatment for 4 weeks in steroid naive patients resulted in a significant improvement compared to CFC-BDP regarding lung attenuation on CT and less increase in air trapping after methacholine provocation, although clinical effectiveness was similar. [58] Another study comparing 400 µg of HFA-BDP by breath activated inhaler to FP 500 µg Diskus resulted in similar decreases in trapped air after 3 months of treatment.[59] Although careful inhalation in this study was stressed, there is no information on inhalation flow rate and breath hold, both important determinants of peripheral deposition.

In a technetium (Tc)-labelled HFA-BDP study with the AeroChamber Plus®, a slow maximal inhalation followed by a 5 - 10-s breath-hold resulted in an increased lung deposition compared with 5 tidal breaths in children from 5 to 17 years.[3] However, it is not clear from this study if patients exhaled to residual volume before starting inhalation and at which inspiratory flow, both important determinants for peripheral airways deposition. Children ≥5 yrs of age are now encouraged to use the deep exhalation-single breath inhalation technique with breath hold if possible.[4]

Of children with asthma who were treated for 4 weeks with 400 µg budesonide, the majority had increased peripheral deposition in a scintigraphic study after a month of treatment compared to the start of the study, suggesting improvement of airway patency and thus increased peripheral deposition.[60] A lung deposition of over 50% with a high peripheral deposition was demonstrated for the small particle ciclesonide on both 2-dimensional and 3-D radionuclide imaging studies.[61] The use of a breath-actuated device or pMDI with HFA-BDP, which has the advantage of both small particle size and lower plume velocity with proper inhalation technique, resulted in a lung deposition of over 50%.[62] In many radionuclide labeling studies, there is no comparison with another drug or device.

Other methods of determining lung deposition as a proxy for clinical effectiveness in CF

Deposition based on clinical studies

Studies on inhaled aminoglycosides[63], aztreonam[64], DNAse[65] and hypertonic saline[66] have primarily focused on clinical effectiveness instead of deposition and have shown beneficial clinical effects. The newly developed Tobramicin Inhalation Powder (TIP), using large porous particles has shown to be non-inferior to nebulized tobramicin.[67-70]

A trial on large airway versus small airway deposition of inhaled DNAse using the AKITA device by adjusting particle size, breathing pattern and timing of aerosol bolus resulted in significant improvement of FEF 75% in both groups within 4 weeks of switching from a conventional nebulizer system to a so called smart nebulizer.[25] Both groups (targeting the small airways in the one group and the larger airways in the other group) improved, probably as a result of increased pulmonary delivery. Further, improvement may also be caused by increased adherence due to the effect of being included in a clinical trial.

Deposition based on pharmacokinetic studies

The advantage of aerolized tobramycin over intravenous administration is that higher sputum levels may be obtained with low systemic concentrations. The sputum concentration can reach levels that exceed the minimal inhibitory concentration (MIC) of the *Pseudomonas aeruginosa* isolates by 25 times in 95% of patients with an estimated systemic bio-availability of 11.7% for a 300 mg dose.[71] Although the sputum concentration may be high in the upper airways, there will definitely be a concentration gradient of aminoglycosides towards the lung periphery with a factor 25, possibly resulting in a level just above MIC in the peripheral airways *(chapter 5)*.[14] For aminoglycosides, high peak levels (far above the MIC) are needed to make use of the reported post-antibiotic effect of this class of drugs. Urinary excretion of aminoglycosides has been shown to reflect lung deposition.[72]

Deposition based on radionuclide labeling with gamma scintigraphy

Gamma scintigraphy can be used to compare lung deposition between different drug formulations or devices. For example, efficient deposition for the TIP powder was demonstrated using gamma scintigraphy in healthy volunteers and compared to pharmacodynamic measurements.[16] Consistent with the pulmonary deposition data, twice as much tobramycin reached the systemic circulation using the TIP powder compared to nebulized tobramycin. In another study, gamma scintigraphy demonstrated 40% less lung deposition of nebulized tobramycin when nebulized with the eFlow® rapid compared with LC Plus® nebulizers in patients with CF.[22]

9.7 Myths in inhalation technology: the big 7

The translation of aerosol and deposition studies to everyday clinical practice has shown to be difficult for clinicians and several myths exist in inhalation technology. The 7 most important myths will be unravelled here.

First, as much emphasis has been placed on fine particle dosing, many physicians believe that 'the smaller the particle, the better'. However, this is not correct as particles < 1 µm are mostly exhaled again. Although fine particle aerosols from HFA pMDIs may lead to an improvement of deep lung deposition, this is not only because their particles are smaller, but also because of the lower plume velocity from many HFA driven pMDIs compared to the older CFC driven pMDIs. Both smaller particles and lower release velocity lead to less upper airway impaction and therefore, the effect of small particles can not be attributed to particle size alone.

Another myth is that doctors presume that a homogeneous lung deposition can be reached with inhalation. Considering the most optimal drug distribution over the entire lung that can be achieved (approx. one third in the upper [generation 0-11], one third in the intermediate [generation 12-16] and one third in the peripheral [generation 17-23] lung)[15-17] and the exponentially increasing lung surface from the trachea to

the alveoli, the drug concentration will decrease by at least a factor 25 from generation 0 to generation 23 *(chapter 3)*.[14]

Third, in general it is believed that the mass median aerodynamic diameter (MMAD) of an aerosol, is the most important characteristic of an aerosol. However, the MMAD does not give any information about it's size distribution, nor about the mass fraction of the dose for which the MMAD is given. Fine particle doses are only a fraction of the label claim and MMAD does not reveal which fraction. Besides, DPIs do not have a single MMAD. Aerosols from DPIs have a bimodal distribution, one for the drug bound to carrier and one for the drug released from the carrier. The aerosol from a DPI is therefore a mixture of (small) drug and (large) carrier particles with drug particles still attached to their surface and the distribution curves of both particles may show overlap. If an MMAD for a dry powder aerosol is given, it has to be defined for which size fraction of the particles in the aerosol and which mass fraction (compared to label claim).

Fourth, a flow rate independent fine particle fraction is considered important.[73] However, deposition shifts to larger airways with increasing flow rate. To obtain the same central and deep lung deposition, more fine particles need to be released with increasing inspiratory flow and preferably these particles have to be finer too.[26]

A fifth myth is that a high resistance device would require a larger amount of work than a lower resistance device. High resistance inhalers generally have highly effective dispersion principles. They deliver the same fraction of the dose as fine particles at the same approximate pressure drop (within the range 2-4 kPa) as low resistance devices. However this pressure drop across a high resistance DPI results in a much lower air flow rate compared to a low resistance DPI, which reduces mouth and throat deposition. When the air flow rate is low, pressure equilibrium between the lungs and the ambient air takes longer than when the air flow rate is high. This enables the patient to generate a higher pressure drop across the higher air flow resistance and to continue this high pressure drop over a longer period of time. When the resistance is low, the air flow rate is high and inhalation time thus short. This philosophy is illustrated in the paragraph "Message in a bottle" *(chapter 1)*.

Next, a common misunderstanding is that the use of spacers always increases lung deposition compared to deposition from pMDIs without spacer. In fact, spacers only decrease upper airway deposition and thus, reduce local side effects. Additionally, they help to overcome coordination problems. Depending on the inhalation manoeuvre, lung deposition may be increased or decreased with a spacer in comparison with a pMDI without a spacer, according to the design of the spacer, the aerosol formulation, the patient's inhalation technique and the extent to which the electrostatic charge within the spacer is controlled.

Last, although organizations as the Dutch Asthma Foundation and scientific societies as the European Respiratory Society[4] promote uniform instruction for each type of device, we want to make clear that this may not always be correct.[26] For example, low resistance DPIs with a flow rate independent fine particle fraction should not be used while inhaling forcefully, as this will shift the deposition from the deep lung towards larger airways. For such inhalers it is better to confine to a pressure drop of 2-4 kPa across the device. For high resistance devices, which do not facilitate high flow rates, this is less critical, particularly when they also release more fine particles at higher flow rates.

9.8 What remains to be done: future perspectives

Increased skills of the health care professional, improved patient adherence, newly developed devices and drug formulations may all change pulmonary drug deposition and clinical effect for the better *(Figure 9.1)*.

Although the benefits of inhalation treatments are clear from both a clinical and scientific point of view, in clinical practice this may not be the case from a patients perspective. Encountered difficulties with young children may be poor cooperation, even smaller airway diameters, poor inspiratory performance and poor understanding of the instructions given. Adolescents are a challenge particularly in increasing adherence. Further, administration of inhaled drugs may be time consuming and spacers are not easy to take with you while travelling or attending school. For all these reasons, inhalation treatment may be difficult to adhere to. The voice of the patient and caregiver should be heard in order to improve treatments by making them faster and easier to administer. As the patient is the one with the disease (Law number 4 in "The House of God")[74] devices as well as instructions should be easy and clear with as few doses a day as possible to increase adherence.[75]

Additionally, improvement of the knowledge of the health care professional is desired. In practice, many doctors, nurses and clinical researchers believe that the MMAD is the most important parameter determining where drugs will be deposited, but this view is very incomplete. The inspiratory flow manoeuvre is equally important in determining the site and extent of drug deposition. The severity of the disease in the various airway generations and airway calibre also influence deposition pattern. Furthermore, most clinicians do not know that large differences exist in (fine-particle) output between various pMDIs and pMDI-spacer combinations, DPIs and nebulizer compressor systems *(chapters 3, 4, 6-8)*. It is therefore important to have a balanced view on the many variables and their interactions *(Figure 9.1)*, as they all determine the amount of drug and the location of drug deposition in the airways.

It is possible that in the past, inadequate delivery systems for inhaled drugs have hampered clinical success. We provided suggestive evidence for this statement regarding inhaled insulin *(chapter 8)*, but others have suggested that inadequate delivery systems might have played a role also for the failure of inhaled disodium cromoglycate[76], which is still used successfully for allergic conjunctivitis. For nebulized drugs and high dosages delivered by DPI, faster administration may be important, especially when many drugs need to be used as in cystic fibrosis ("so many drugs, so little time").[77]

An ideal DPI should preferably have a high internal resistance with a flow dependent increase in fine particle dose to compensate for the higher impaction in the upper airways with increased flow rates. The resistance should not be too high on the other hand, because of insufficient energy for proper dose release and dispersion. Besides, at very low flow rates with too high resistance, the inhalation time from residual volume to total lung capacity may be too long, leaving no time for a breath hold pause. Further, the dose range for which the same inhaler can be used should ideally be expanded, especially for systemic delivery and preferably as a single inhalation. For relatively low dose drugs, as ICS and bronchodilators that are given in 100-1000 microgram range, current DPIs do their job well enough. For drugs given in high doses, as antibiotics (given in a 100-200 mg range) as well as for drugs needing systemic delivery (insulin, also given in 1-10 mg range) more effective DPIs are needed with effective dispersion, allowing for high doses to be inhaled with a single inhalation and release of the drugs early during inspiration.

Developing new formulations which need to be administered once daily may improve patient adherence.[75] Likewise developing DPI formulations instead of nebulization fluids may increase adherence to treatment. However, the Tobramycin Inhalation Powder (TIP), developed to save time in patients with CF, when compared to inhalation of tobramycin solution, requires the inhalation of four capsules twice a day, which only halves the administration time compared to current smart nebulizers.

Improved inhalation therapy will further improve by inhaling the required dose with one inhalation. We have shown that this is feasible for insulin *(chapter 8)*. Lower fine particle dose variability is especially important in drugs with a narrow therapeutic index such as insulin. Monodisperse fine particle aerosols could be a way forward, but unfortunately they can only be generated in low concentrations in wet aerosols. For systemic delivery a relatively large dose would be needed to reach the desired effect.
The benefits of liposomal drugs with controlled release[78] could lead to a lower dosing frequency probably increasing adherence.[75]

Drug formulations may contain additives, which preferably should not be inhaled. Indeed the development of a conservative free tobramycin solution has been related to reduced side effects and increased tolerability.[79] The upcoming Afrezza® insulin also contains many additives to create optimal aerodynamic characteristics, but as we have shown with the Exubera® insulin formulation consisting of large porous particles, lung delivery can also be improved by using more effective inhalation technology *(chapter 8)*.

Then, although a high fine particle dose might increase clinical effects, in vivo studies could not prove this. The ideal study to compare effects of large and small fine particle ICS doses from pMDI- valved holding chambers combinations would include 1) determination of fine particle output under known in vitro conditions as inspiratory flow rate and application of a breathhold 2) randomized controlled trials of longer duration, with 3) preferably 2 doses per ICS and 4) recording of humidity, inhalation manoeuvre (preferably a single inhalation after exhalation to residual volume followed by a 5-10 s breath hold) and 5) focus on important clinical endpoints as asthma control, symptom free days, exacerbations, doctors visits and hospital admissions. It may be expensive and difficult to take all these factors into account, but all have an impact on outcome. Preferably, studies should not have a non-inferiority design as new drugs and devices should be superior in either efficacy or convenience of use.

In many studies comparing different ICS with DPI's, pMDI-VHC combinations or nebulizer systems, it is usually not only the effect of the 2 drugs that are compared, but also the effect of 2 delivery systems (e.g. a high and a low internal resistance DPI or a HFA- and CFC-pMDI). The study results are thus only valid for the drug-dose-device combinations investigated and using another device or dose comparison might yield other results.

Study results in children should be reported separately from those in adults, as their airway diameters differ from adults. Smaller airway diameters are likely to change particle deposition, as has been decribed when patients with asthma were compared to healthy controls.[80] This change in deposition may well be caused by enhanced flow velocity and turbulence in smaller airways, especially when airway diameters are further reduced by bronchospasm, inflammation and/or mucus hypersecretion.[23]
Optimal pulmonary drug delivery depends on patient related factors (age, disease, adherence), device related factors (fine particle dose), drug related factors and environmental conditions (humidity for spacers, jet flow for nebulizers). Clear communication and instruction is therefore essential to further

improve treatment. The patient should be central in this approach and should be involved in the decision making process.[81-83] It is essential in a patient centered care approach with shared decision makingto explicitly take into account patient circumstances and preferences.[84]

9.9 What have we learned

A high fine particle fraction (1-3 µm) inhaled with a slow inhalation, followed by a breath hold pause is optimal to treat diseases that affect the entire airway, like asthma, COPD and CF. The alveoli need to be reached for systemic absorption, which requires the same inhalation manoeuvre. The studies in this thesis stress the importance that in vitro data from drug-device combinations should be used in interpreting clinical studies and also pave the way for studies taking environmental conditions such as air humidity into account.

Taken together, in this thesis we have shown that higher relative air humidity leads to a relevant increase in fine particle output from pMDI-spacer combinations compared to lower air humidity *(chapter 4)*.
Second, as a proof of principle, a larger fine particle fraction obtained with improved inhaler technology that facilitates an appropriate inspiratory manoeuvre for deep lung deposition, including a longer breath hold pause, results in increased systemic availability of insulin *(chapter 8)*. This may open avenues for the development of vaccines and treatment by inhalation for many other diseases including tuberculosis.
Third, besides the driving flow of a nebulizer *(chapter 7)*, the patient compliance with cleaning instructions has a huge influence on the drug output. Despite clear and repeated instructions it is difficult to adhere to time consuming regimens *(chapter 6)*. To improve adherence, shared decision making and patient centred communication skills are essential and the expression "drugs don't work in patients who don't take them" will always be true.

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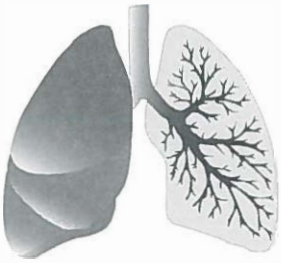
APPENDIX



Separated by a common translation? How the British and the Dutch communicate

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SUMMARY

The British and Dutch share a long naval-, war- and medical history, in good times aswell as bad. Their language has a common Germanic origin, but the English people may use special ways to express values or opinions, from which the sometimes paradoxical meaning is not always clear to the other party. Particularly with the Dutch, renowned for their directness, this may cause confusion. We provide a comprehensive set of expressions, each with paired interpretations, to foster Anglo-Dutch cooperation.

Editor's Note:

From time to time (Canadian translation for rarely or never) I feel it is important to publish an article that will further international relationships and clarify scientific discourse. The following paper is an example of such a publication. It is quite clear that in certain parts of the world hockey seems to have been supplanted by the sinking of ships and football.

—Victor Chernick

INTRODUCTION

The British and Dutch share a long history, in good times as well as bad. In the Anglo–Dutch wars, Michiel Adriaenszoon de Ruyter played a major role as a skilled Dutch admiral as did his famous British counterpart Admiral Robert Blake. Nowadays the Royal Navy and Royal Dutch Navy still cooperate together at high level in the Flag Officer Sea Training. Our health systems differ, but also share common ground: the Dutch equivalent of the National Health Service is a compulsory health insurance system for everyone. British and Dutch doctors meet in conference and on other occasions; scientific articles are written together and reviewed by both nationalities. Medical care is strongly influenced by centuries of development of cultural beliefs and ideas, as has been beautifully written up by (the deceased) Lynn Payer.[1] Differences between the two nations exist in many areas, although for the outside world the Dutch and the British seems to get along quite well. Despite the differences in the success or otherwise of their respective football teams.[2] the Dutch and British have a lot in common besides the weather.
The medical communities of both countries have a significant profile in preclinical and clinical research. Language- and cultural differences may play an important role at conferences and in the process of submitting and reviewing articles. The aim of this article is to improve mutual understanding during these professional and social encounters as well as in the writing and reviewing process of scientific articles. We hope to show that the provision of a comprehensive set of expressions, each with paired interpretations, fosters Anglo–Dutch cooperation.

MATERIALS AND METHODS

In-depth interviews with Dutch expats and their British employers on common expressions and their meanings resulted in "misunderstandings" that were checked and cross checked for meaning and interpretation. In the following years these expressions have been tested on multiple occasions. Editorial comments and those of reviewers of medical scientific articles were picked out by the authors on the basis of suspected paradoxical meaning and discussed among the authors.

RESULTS

The results from the in-depth interviews and reviewers and editorial comments are posted in tables with the expression itself, it's meaning and how it might be understood by non-British citizens. Although we share a rich history and ideas, there are nevertheless often cases where the communication between the two nations is not as straight forward as might be expected. This is clear from reading *Tables 1 and 2* for social encounters and the reviewing process respectively.

What the British say	What the British mean	What the Dutch understand
I hear what you say	I disagree completely	He[a] accepts my point of view
You must come by for dinner sometime	Just being polite; Goodbye!	He will invite me for dinner in the course of time
Very interesting	I don't agree	He likes my idea
With the greatest respect	You must be a fool	He respects me/my view
I'm sure it's my fault	It's your fault!	It is his fault
That is an original point of view	You must be crazy	They like the idea
I almost agree	I don't agree	He almost agrees
You'll get there (eventually)	No way you will make it	Encouragement to go on
I'll bear it in mind	I won't do anything about it	He will use it when appropriate
Could we consider some other options	I don't like your idea	He is still in the process of thinking
I would suggest	Do it as I want you to	An open suggestion
By the way	The primary purpose is	Not very important
Perhaps you could give this some more thought	Don't do it, it's a bad idea	Consider possible road blocks
Quite good	A bit disappointing	Quite good
Not bad	(very) good	Average or poor

[a] Where "he" is mentioned "she" can also be read.

Table 1 *Translation Guide Social*

DISCUSSION

From these study results is it is clear that the British use of language often causes confusion among the other nationalities. English people may use special ways to express values or opinions, from which the sometimes paradoxical meaning is not always clear to the other party. Particularly with the Dutch, renowned for their directness, this may cause confusion. We hypothesize that the differences may well have their origin in the gentlemanlike, polite nature of the British (according to the Dutch authors) or their utter dishonesty (the British version). It has been shown that British gentleman-like behavior is often even maintained in disastrous circumstances such as the sinking of the Titanic (April 15th 1912), where British nationality overall (corrected for class of travel) resulted in lower probability of survival ($P<0.01$) compared to other European nationalities and US citizens.[3] Time is of importance however, and a more slowly sinking ship may lead to a different view: the Titanic sank in 2 hr 40 min, the Lusitania in just 18 min; the latter leading to more selfish behaviour.[4] This guide may helpful not just to British and Dutch medical doctors, but also to native speakers from other European languages and even those using American- English or Australian-English. Both Dutch and English have developed from the Germanic languages [http://en.wikipedia.org/wiki/English_language]. This can be illustrated by the conjugation of verbs (present/past/past participle): "eten/at/gegeten" and "to eat/ate/eaten." Nevertheless, the possibilities for misunderstandings are numerous, as we show in *Table 1* for social encounters, and in *Table 2* for the scientific review environment. As a rule of thumb, most Dutch people are less used to playing with the language as the British. This contrasts with the much greater levels of Dutch footballing schools.[2] Both may be just as true for German, Spanish, and other European nationalities. However, eloquence on the football field and in the use of language seem to be two different things. This guide should at least help people to get to grips with the latter. The former we will leave for another year when we can all talk about football again without the relative recent memory of a world cup.

What the British write	What the British mean	What the Dutch read
Please consider	Do it or forget it	He[a] leaves it up to me
I have a further suggestion	Take it or leave it	He leaves it up to me
The method described is rather original	Bullshit	It's a good method
I have a few preliminary suggestions	I strongly suggest you to follow my suggestions	Don't change anything until final suggestions have been made
Reads well	Really good	Average
I am somewhat disturbed by the methodology	I am crossed	He is not feeling too comfortable about it
With all due respect	You don't know what you are doing, I have a better suggestion (polite disagreement)	With the greatest respect
A few issues that need to be addressed	A whole lot needs to be changed	2–3 issues need rewriting
An issue that worries me slightly	A great worry	A minor issue
I am sorry we have to reject your paper on priority grounds	Your paper sucks	My paper nearly made it
I am sorry to disappoint you on this occasion	I could not care less	He is sorry

[a] Where "he" is mentioned "she" can also be read.

Table 2 *Translation Guide: Professional (Review Process)*

BACKGROUND AND CONTRIBUTIONS

Prof. A. Bush is the 2008 Jonxis medallist; his prize lecture "Asthma: Problematic, Difficult, and Therapy Resistant-the Brompton experience" in 2008 was completely eclipsed by BR's presentation of some of these ideas. NR developed *Table1* of the translation guide on the basis of her work with Dutch expats.[5] BR has tested elements of this translation guide on different occasions with British medical doctors, which always resulted in a good laugh as these elements are generally believed to reflect the true meaning. The naval inspiration results from fulfilling the military service as a ships doctor for the Royal Dutch Navy, where Dutch and British doctors did get along well. AB added even more intercultural flavor to the translation guide and reviewed the English. The reference to football was initiated by the World Cup tournament in July 2010.

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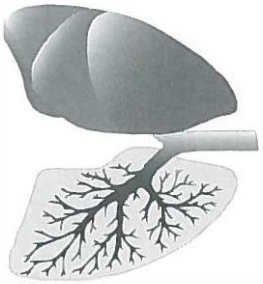


SUMMARY

The general aim of this thesis is to improve inhalation treatments for pulmonary and non-pulmonary diseases. In this thesis issues in inhalation therapy in asthma and chronic obstructive pulmonary disease (COPD), both being highly prevalent diseases which are treated with inhaled corticosteroids (ICS) and inhaled bronchodilators, are adressed. Patients with cystic fibrosis (CF) frequently use inhalation therapy with inhaled antibiotics and mucolytics. Newer areas or areas under research for inhalation treatments include tuberculosis, the administration of vaccines (influenza, measles) and the inhalation of chemotherapeutic drugs in lung cancer, immunosuppressants after lungtransplantation, vasodilators in pulmonary hypertension and insulin in diabetes.

In this chapter the major research findings from this thesis are summarised. The chapter starts with a short introduction into particle and fluid dynamics in the airways and deposition mechanisms for inhaled aerosols, which are suspensions of solid particles or liquid droplets in a gas. Specific diseases and their targets for inhaled drug treatment will be shortly discussed and an overview of the techniques used in this thesis is presented. All these topics are covered in more detail in *chapter 1*.

Most inhaled drugs work locally, but drugs like insulin and vaccines need to be absorbed in the large, highly perfused alveolar surface area to have a systemic effect. The advantages of inhaled therapy for local treatment in the lungs are delivery of a high dose in the target area with strongly reduced systemic adverse side effects when compared to oral or intravenous treatment. The advantage of vaccines, e.g. against childhood diseases, in a powder formulation for inhalation are that for administration no needles are required. Subsequently there is no need for disposal of needles, which reduces infection risks. Moreover, vaccines in powder formulation for inhalation can be stored at room temperature instead of in the refrigerator.

Despite many advantages, inhaled therapy is not that simple. Drug deposition in the lung after inhalation depends on a variety of factors such as the type of disease, disease severity, the airway geometry depending on age, the particle size distribution of the drug and the inhalation manoeuvre.

The bronchial tree is a branching system starting with the trachea (generation 0), which divides into a main stem bronchus for each lung (generation 1) and then continues to branch by dichotomy like a tree, progressively reducing the airway diameters with each new generation. From the trachea to the alveoli there are 23 generations and consequently 2^{23} branches. The diameter decreases from 18 mm for an adult trachea to less than 0.5 mm in the preterminal bronchioli, just before the alveoli. The number of airways within each generation increases faster than the airway diameters decrease, which results in an exponentially increasing total cross sectional area towards the alveoli and a concurrent decrease of the air flow velocity and thus, velocity of the aerosol particles. This decrease in velocity is important for the drug deposition mechanisms in the airway. Similar to the exponential increase in the total cross sectional area, the surface area were drugs can be deposited also increases exponentially *(Figure 1)*. The last 7 airway generations make up for 95% of total lung surface area.

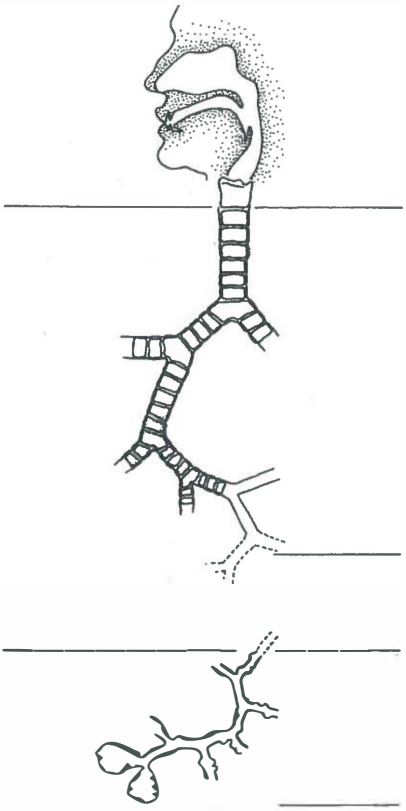


Figure 1 *The branching airway system consists of 23 generations with an exponentially increasing surface area towards the peripheral airways.*

Upper airways

Airway generations 0 -11: conducting airways
1% surface area

Airway generations 12-16: transitional airways
4% surface area

Airway generations 17-23: respiratory airways or peripheral airways
95% surface area

For efficient deposition in the central and peripheral airways, particles with an aerodynamic diameter of less than 5 µm are needed. The preferred size range is rather in the range between 1 and 3 µm. Particles that are larger than 5 µm deposit primarily in mouth, throat and upper airways, whereas 40-50% of particles smaller than 1 µm is exhaled for a large extent, even after a breath-hold of several seconds.

Mostly particles in an aerosol have different sizes. Wet aerosols, as released from a pressurised metered dose inhaler (pMDI) or a nebulizer system are usually spheric (round). Most devices release aerosols in a size range form 0.1-10 µm. Moreover, dry aerosol particles may have different shapes and densities. Therefore aerosols cannot be compared on size only as the aerodynamic behaviour of aerosol particles depends on size, shape and density. The sizes of irregularly shaped particles can be compared with each other using the equivalent volume diameter (D_E) *(Figure 2)*. Particles with the same D_E can aerodynamically behave differently when they have different shapes and densities. Particle shape can be expressed using the dynamic shape factor (χ) which equals 1 for a sphere, whereas $\chi > 1$ for irregular particles. Usually particle density (ρ) is given in g/cm^3. The density of water is 1 g/cm^3, whereas the density of solid dry particles is mostly larger than 1, unless they are extremely porous. To standardise for these variations in shape and density, the aerodynamic diameter (D_A) is frequently used *(Figure 2)*.

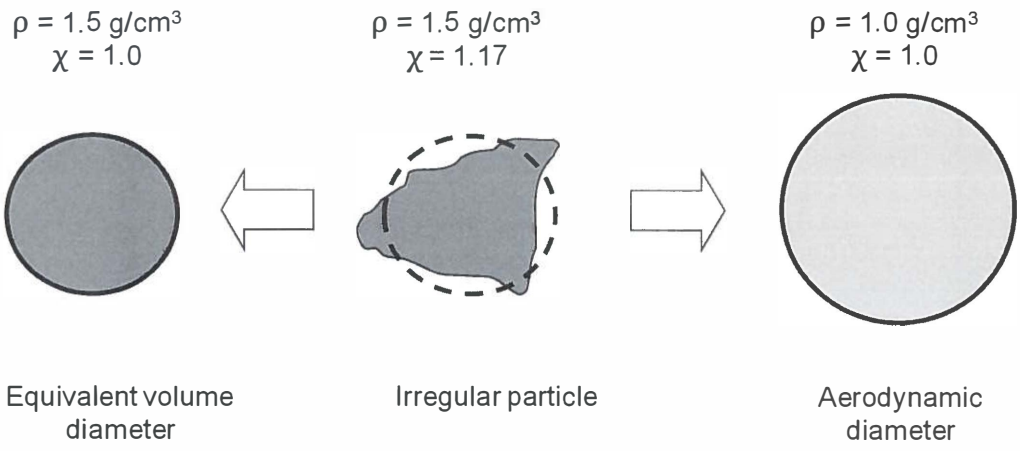


Figure 2 *Methods to characterise irregularly shaped particles in the aerosol technology. D_E is the equivalent volume diameter: the diameter of a sphere (round particle) having the same volume as the irregular particle in consideration. D_A is the aerodynamic diameter, defined as the diameter of a sphere ($\chi = 1$) with the density of water ($\rho = 1$ g/cm³) having the same settling velocity in still air. Particles with the same D_A have the same aerodynamic behaviour. $D_A = D_E\sqrt{(\rho/\chi)}$*

Most aerosols contain particles within a certain size range and are called therefore called polydisperse aerosols. Polydisperse aerosols are usually characterized by measuring the particle size distribution. Particle size distributions are usually derived from a mass, volume or number distribution curve subdivided in diameter classes. Within each diameter class, the contribution of particles to total number, volume or mass can be computed and graphically depicted. From this cumulative distribution graph, a median diameter can be read. Despite the fact that most aerosol distributions are skewed (not log-normal), aerosols are usually compared using median diameters. The median diameter is the midpoint diameter; 50% of the volume, mass of number is in coarser particles and 50% is in finer particles. For a distribution curve based on volume, as measured with laser diffraction analysis, the median diameter is the volume median diameter (VMD or X_{50}). In the same way, from a cumulative mass distribution curve the mass median diameter (MMD) can be read. When the mass distribution is measured as a function of the aerodynamic diameter, this enables to characterise these aerosols with their mass median aerodynamic diameter (MMAD). For spherical droplets with unit density (as from diluted aqueous solutions), the VMD measured by laser diffraction analysis equals the Mass Median Aerodynamic Diameter (MMAD). Particles with the same aerodynamic diameter, whether large and porous (such as Exubera® dry powder insulin or Tobramycin Inhalation Powder) or spheric and irregularly shaped, will aerodynamically behave in the same way and reach the same target areas with the same inspiratory flow manoeuvre. Despite the emphasis many clinicians place on the importance of MMAD, *chapter 1* explains why MMAD alone is not enough to characterize an aerosol.

Aerosol generation devices can be divided into four different groups depending on their principle of operation: pressurized metered dose inhalers (pMDIs), dry powder inhalers (DPIs) nebulizers and soft mist inhalers. In childhood, pMDIs are always combined with a valved holding chamber (VHC) or spacer to overcome coordination problems and to decrease local side effects. DPIs usually contain micronised drug

blended with larger (lactose) carrier particles. The drug needs to be dispersed (released) from the carrier during inhalation. A more forceful inhalation usually results in higher fractions of particles released from the carrier which may also be smaller with the possibility of deeper lung penetration. Nebulizers convert drug solutions or suspensions into an aerosol by either a jet stream generated by a compressor (a nebulizer-compressor system) or by means of high frequency vibration applied to a perforated membrane in contact with the drug solution (mesh nebulizer).

The combination of aerodynamic particle size and inspiratory flow determines the site of deposition. The aerosol speed in entering the airways depends on the forcefulness of the inhalation manoeuvre, the inhaler resistance to air flow and the speed with which the aerosol is released from the device. Usually the patient's inhalation manoeuvre and the inhaler resistance are the most important determinant of the three, except for pMDIs. The appropriate inhalation manoeuvre depends on the device. Nebulizers release an aerosol in a 5-15 minute time frame, and the aerosol is inhaled with tidal breathing. An aerosol from a nebulizer system may reach all airways by diffusion (mixture) taking place during the exchange of inhaled and exhaled air. Some dry powder devices may have a short emission time enabling inhalation of the entire dose in a single inhalation. In general, the optimal manoeuvre to reach the peripheral airways is exhalation to residual volume prior to an inhalation to total lung capacity followed by a 5-10 second breath-hold. A breath-hold increases deposition by sedimentation.

Aerosol particles can be deposited on the airways by three mechanisms: impaction, sedimentation and diffusion. Impaction is the 'crashing' of aerosol particles on the walls of an airway conduit. This is either due to high velocity, high particle mass or both, resulting in a particle following its own straight track instead of following the carrying airstream in bends and bifurcations of the airways. To keep the drug losses due to impaction in mouth, throat and the first airways bends to a minimum, inhalation should not be too fast.
Sedimentation is particle settling by the force of gravity. Sedimentation mainly occurs in the central and peripheral airways, where the air velocity is much lower and distances to the airway wall are shorter. The settling velocity increases with the second power of the particle diameter. Sedimentation is a time dependent mechanism of deposition and the efficacy of particle settling by sedimentation may thus be increased by a longer residence time of particles in the airways by a breath-hold.

The site in the lungs where drugs are needed (target area) generally depends on the localisation of the disease process and the localisation of the receptors for a specific drug.

The inflammatory process in asthma involves all airways and in particular the peripheral airways. ICS are considered the most powerful anti-inflammatory treatment modality in asthma *(chapter 2)* and COPD. ICS receptors are present in all airways, but their density increases towards the peripheral airways. Therefore, airway generations 7-23 are the main target for ICS. Bronchodilators in asthma and COPD aim at smooth muscle relaxation to counteract the bronchoconstriction caused by smooth muscle contraction. Smooth muscle is mainly present around the conducting and transitional airways, The airways with smooth muscle but without protecting cartilage are airway generations 7-14. Thus, the target area for bronchodilators (generations 7-14) is more proximal than that for ICS (generations 7-23).

In cystic fibrosis, both bugs and mucus are present in all airways and therefore, all airways are the target area for inhaled antibiotics and mucolytics.
The optimal target area within the lungs for the delivery of drugs to the systemic circulation (e.g. inhaled insulin) is the alveolar region, where the barrier for absorption is thin and only minimal mucociliary clearance occurs.

Chapter 2 reviews current anti-inflammatory treatment in asthma and makes clear that ICS are still the mainstay of maintenance treatment. With the inflammatory process being present in all airways with emphasis on the small airways, as well as the density of ICS receptors increasing towards the periphery with an exponentially increasing airway surface area, the peripheral airways are the main target area for ICS.

In chapter 3 the particle size distributions of aerosols from the most commonly used ICS-pMDIs in the Netherlands are compared with each other. HFA-propellant based pMDIs only deliver the aerosol at a low plume velocity (compared with the -now phased out- CFC-pMDIs) when having ethanol as a co-solvent. An aerosol with a smaller MMAD does not always contain a larger fraction of ICS in the relevant particle rage of 1-3 µm, as these aerosols may also contain more submicron particles (< 1 µm) that are exhaled to a large extent. Of note, there was a significant decrease in delivered dose during lifespan for fluticasone 125 and 250 µg/dose pMDIs. The large variations in performance that were found over pMDI life span and delivered fine particle fraction underscore that the in vitro evaluation of ICS pMDIs under well-controlled conditions is mandatory for adequate evaluation of drug delivery studies in patients. The output in small particles in this study was the highest from the ciclesonide and HFA-beclomethasone pMDIs and therefore theoretically, these pMDIs are most suitable for pediatric use.

Chapter 4 deals with several variables that have an impact on the delivered (fine particle) dose from a PMDI with spacer. It is often stated that spacers increase the dose available for inhalation due to selective retention of larger particles (that would be lost in the spacer due to the force of gravity). However, the study in *chapter 4* failed to show such a selective retention of the larger particles. Further, there was no influence of inspiratory flow rate on the particle size distribution from the spacer. However, we would advice against a fast inhalation as this will lead to more drug losses by inertial impaction in the upper airways. The effect of a longer lag time (waiting time between releasing a dose and inhaling it from the spacer) on output up to 20 seconds is considerably less than was found in previous studies. The most important finding of *chapter 4* was that an increase in relative humidity from low (25-35%) to high (75-80%) can result in an up to two-fold increase in drug output from the spacer. From bench to bedside, the efficacy of the therapy might thus be improved by administrating the medication in a humid environment (e.g. a used bathroom). The particle size distribution does not change with increasing humidity, thus a higher output translates directly to a higher fine particle fraction.

In chapter 5 the drug target area in CF is described, in order to advice on the choice of device and inhalation manoeuvre to optimally treat *Pseudomonas aeruginosa*. As airway inflammation, infection and obstruction by mucus in CF is present in all airways, the entire airway should be targeted. With the exponentially increasing airway surface area from 5% for airway generations 0-16 to 95% for airway generations 17-23 *(figure 1)*, it is clear that the peripheral airways should be targeted. It is hypothesised that the conductive airways (generations 0-11) will be treated by the drug 'losses' by (mainly) impaction during inhalation. Even with optimal peripheral targeting (which depends on particle size distribution

and breathing manoeuvre) a 25 fold drug decrease in concentration between conductive and peripheral airways will remain.

Chapter 6 describes the effect of 6 months use of two types of nebulizers in daily practice by patients with CF and the effect of (lack of) adherence to cleaning instructions on their nebulizers' performance. A new nebulizer, the eFlow rapid® became very popular in the CF community before registration studies were performed. The advantages of the eFlow rapid® compared to many nebulizer-compressor combinations are its small size and silent operation. In *chapter 6* it is demonstrated that the eFlow rapid® produces tobramycin aerosols with a VMD of 3.5 µm, which is considerably larger than the VMD of 2.8 µm produced by the Pari LC® plus nebulizer with Turboboy® N compressor. As the chance of impaction is related to the second power of the particle diameter, it may be expected that the drug losses in mouth, throat and upper airways are higher for the aerosol from the eFlow Rapid® than for the aerosol from the Pari LC® Plus. The eFlow Rapid® makes use of a vibrating mesh in contact with the drug solution. The oscillation of the mesh forces the drug solution through funnel shaped pores in the membrane, thereby creating an aerosol, Despite instructed not to do so, patients used the eFlow Rapid® to nebulize other described drugs and they only partially adhered to cleaning instructions. This resulted in partial clogging of the mesh and consequently, in a decreased output rate and change in particle size distribution over time. As the used eFlow Rapids® automatically switched off after 10 minutes, this led to a decrease in delivered dose. After the mesh was replaced, the system functioned normally again. It was also found that the VMD of the aerosol from the Pari LC® Plus increases after 6 months use. This study demonstrates that communication between patient and health care provider is important, but does not automatically lead to full adherence.

Chapter 7. Two commonly nebulized antibiotics in CF, tobramycin and colistin are both available in two different formulations. The particle size distributions of the aerosols from these four formulations were compared after nebulization with four nebulizer systems. Each of the nebulizers was tested with four jet pressures ranging from 1-2.5 bar, as it is known that a higher pressure results in a smaller particle size. When tested under the same circumstances (same nebulizer and same jet pressure), all formulations resulted in the same particle size distribution and they are therefore interchangeable. With a jet pressure of 1 bar the VMD varied from 3.5 to 2.5 µm; at 2.5 bar this difference is smaller: 2.2 and 1.6 µm. As explained earlier, based on the impaction parameter these differences can be expected to be relevant for the area of deposition in the airways. Use of the Ventstream® nebulizer resulted in the smallest particle size under all circumstances.

In chapter 8. we describe the use of an insulin dry powder formulation (Exubera®), which is now withdrawn from the market, to treat patients with diabetes. First, we recorded the primary particle size distribution of this insulin formulation by laser diffraction from RODOS dispersion. Then, we determined the mass fraction of fine particles (1-3 µm) of this insulin formulation from two DPIs: the Exubera® inhaler and the Twincer™. Third, we looked at the effect of the dose weight on the particle size distribution of the aerosol from both inhalers. Based on in vitro characteristics, especially the released fine particle dose with optimal inhalation manoeuvre, we predicted the dose from both inhalers that would lead to equivalent systemic insulin availability from both devices. In the Twincer™, half of the Exubera® insulin formulation leads to equivalent blood insulin levels compared to the Exubera® inhaler. This unique experiment demonstrates that the combination of an increased fine particle dose and the appropriate inhalation manoeuvre (exhalation to residual volume, followed by a slow, deep inhalation and a 5-10 second breath-

hold) leads to higher systemic availability due to increased peripheral deposition and absorption. Further, the Twincer™ is capable of releasing the insulin early during inspiration, so the drug can indeed reach the peripheral airways. This concept paves the way for the return of inhaled insulin, but more importantly, it also opens up avenues for the administration of vaccines (e.g. against measles), TBC treatment and local chemotherapy against lungcancer.

Chapter 9 gives an overview of all contributing factors to improved drug delivery to the lungs of which many were covered in this thesis *(Figure 3)*. Myths in inhalation technology are discussed in this chapter and directions for future research are given.

Taken together the choice of a drug-device combination can have a large impact on the delivered fine particle dose. Environmental circumstances may have an impact too: higher air humidity leads to increased fine particle dose from spacers. The fine particle dose as found in vitro was indeed predictive for systemic absorption of insulin.

Reciprocal communication between health care providers and patient is of utmost importance to ensure correct inhalation technique and adherence to cleaning instructions. Many improvements are possible and needed. Communication is not only a key factor between doctors and patients, but also between researchers of various countries. In the appendix it is made clear that, despite a common origin of their languages, the British and the Dutch may attribute a different meaning to the same expression, possibly leading to misinterpretations.

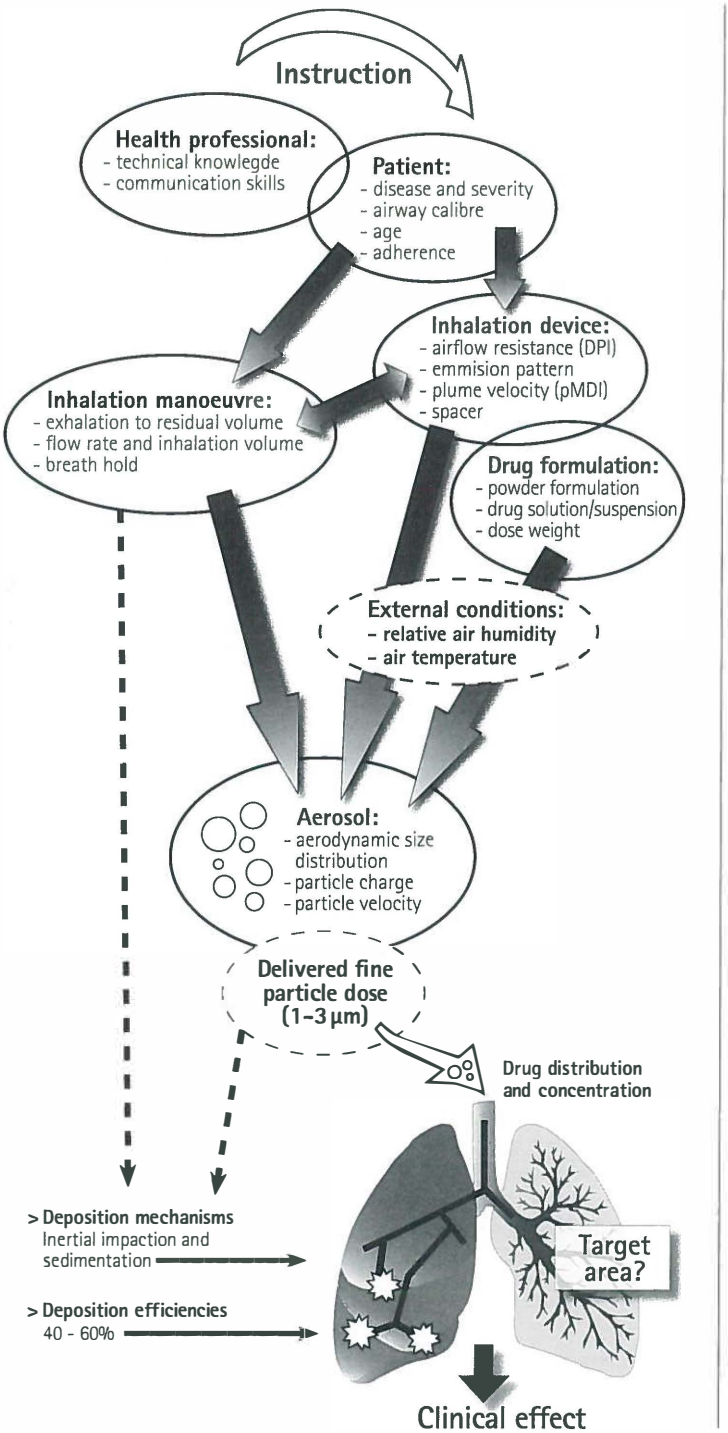
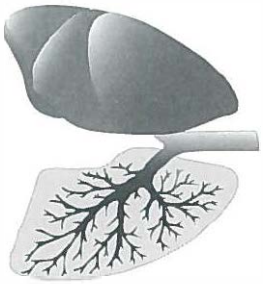


Figure 3 *a mind map of all the drug-device-environmental and human interactions that have an impact on inhaled drug delivery*



SAMENVATTING

Het centrale thema van dit proefschrift is het verbeteren van de toediening van geneesmiddelen aan en via de luchtwegen. De bekendste ziektebeelden waarbij gebruik wordt gemaakt van inhalatiemedicatie zijn astma en COPD (chronic obstructive pulmonary disease, 'longemfyseem'). De gebruikte medicijnen voor deze ziektebeelden zijn met name ontstekingsremmers (inhalatiecorticosteroïden, ICS) en luchtweg-verwijders. Bij Cystic Fibrosis (CF, 'taaislijmziekte') worden vooral antibiotica en middelen om het luchtwegslijm beter kwijt te kunnen raken geïnhaleerd. Nieuwere toepassingsgebieden voor medicijnen in de luchtwegen zijn geneesmiddelen tegen tuberculose (TBC), vaccins, chemotherapeutica bij longkanker, vaatverwijdende middelen bij pulmonale hypertensie (hoge bloeddruk in de long) en insuline bij diabetes mellitus ('suikerziekte').

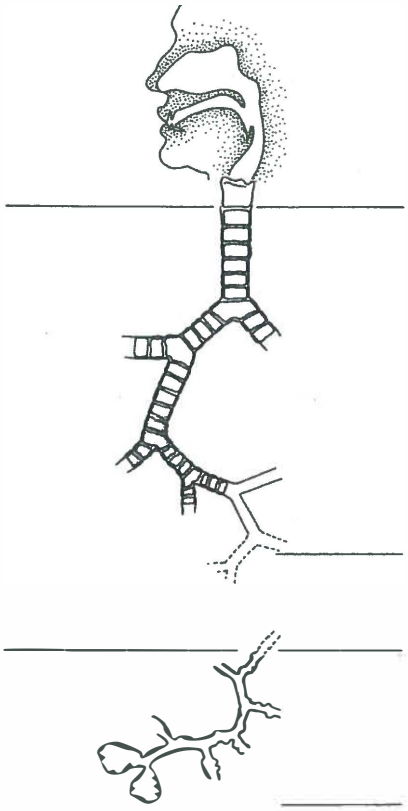
In deze samenvatting worden de belangrijkste onderzoeksbevindingen uit dit proefschrift besproken. Deze bespreking wordt voorafgegaan door een algemene inleiding over de luchtstroming in de luchtwegen en het gedrag van meegevoerde aerosoldeeltjes daarin, die van belang zijn voor transport en afzetting van deze deeltjes. Een aerosol is een mengsel van vaste deeltjes of vloeistofdruppeltjes in een gas. Verder worden de ziekten waarvoor inhalatietherapie veel gebruikt wordt of kan worden, de doelgebieden voor de geneesmiddelen tegen deze ziekten en de toedieningsvormen waarmee de geneesmiddelen in aerosolen kunnen worden omgezet en worden toegediend besproken. Ook wordt een overzicht gegeven van de technieken die voor het onderzoek in dit proefschrift zijn gebruikt. In uitgebreide vorm worden al deze onderwerpen in *hoofdstuk 1* besproken.

Veel van de inhalatiegeneesmiddelen moeten lokaal werken in de luchtwegen, maar in geval van vaccins en het toedienen van insuline gaat het om opname via de longen in de bloedbaan. Doordat de luchtwegen zich steeds verder vertakken en uiteindelijk uitmonden in een netwerk van longblaasjes met een groot oppervlak die in nauw contact met de bloedbaan staan, kunnen medicijnen daar effectief worden opgenomen. Voor de medicijnen die lokaal in de luchtwegen hun werking moeten doen is het voordeel van toediening per inhalatie dat met een veel lagere dosering kan worden volstaan in vergelijking met medicijnen die via de orale (met tabletten of drankjes) of parenterale (met injecties) route worden gegeven waardoor de bijwerkingen ook beduidend minder zijn. Bij vaccins (bijvoorbeeld tegen kinderziekten) is het een groot voordeel dat deze als poeder lang houdbaar zijn buiten de koelkast en zonder naalden kunnen worden toegediend. Dit kan voor veel ontwikkelingslanden een voordeel zijn. Verder zijn naalden een potentiële bron van besmetting en moeten deze na gebruik veilig worden afgevoerd.

Inhalatietherapie heeft dus veel voordelen, maar een effectieve toediening aan of via de luchtwegen is afhankelijk van veel factoren. Die factoren zijn onder andere het ziektebeeld, de geometrie van de longen afhankelijk van de leeftijd van de patiënt (kind of volwassene), de deeltjesgrootteverdeling van het geneesmiddel en de manier van inhaleren.

De luchtwegen vertakken zich van boven (trachea) naar beneden (alveoli). Iedere vertakking wordt een bifurcatie genoemd omdat uit elke luchtweg steeds twee nieuwe ontstaan, resulterend in een nieuwe generatie. In totaal zijn er van de trachea (generatie 0) tot aan de alveoli globaal 23 generaties en daarbij neemt het aantal vertakkingen toe tot 2^{23}. Ook neemt de diameter van iedere volgende generatie af van globaal 18 mm (volwassen trachea) naar minder dan 0.5 mm in de voorlaatste bronchiole, vlakbij de alveoli. Het aantal luchtwegen per generatie neemt echter sneller toe dan de diameter afneemt, waardoor de totale doorsnede voor luchtstroming exponentieel toeneemt richting alveoli en de snelheid van de lucht en de daarin meegevoerde aerosoldeeltjes naar rato afneemt. Dit is van belang voor de mechanismen

waarmee de aerosoldeeltjes in contact worden gebracht met de wanden van de luchtwegen. Naast de toename in doorsnede voor stroming richting de alveoli, neemt tegelijk ook het oppervlak toe waarop de aerosoldeeltjes worden afgezet *(Figuur 1)*. De laatste 7 luchtweggeneraties vormen samen 95% van het totale oppervlak van de longen.



Figuur 1 *De luchtwegen opgebouwd uit 23 generaties met toenemend oppervlak voor stroming en afzetting van aerosoldeeltjes.*

Bovenste luchtwegen

Generaties 0 -11: hogere luchtwegen
1% oppervlakte

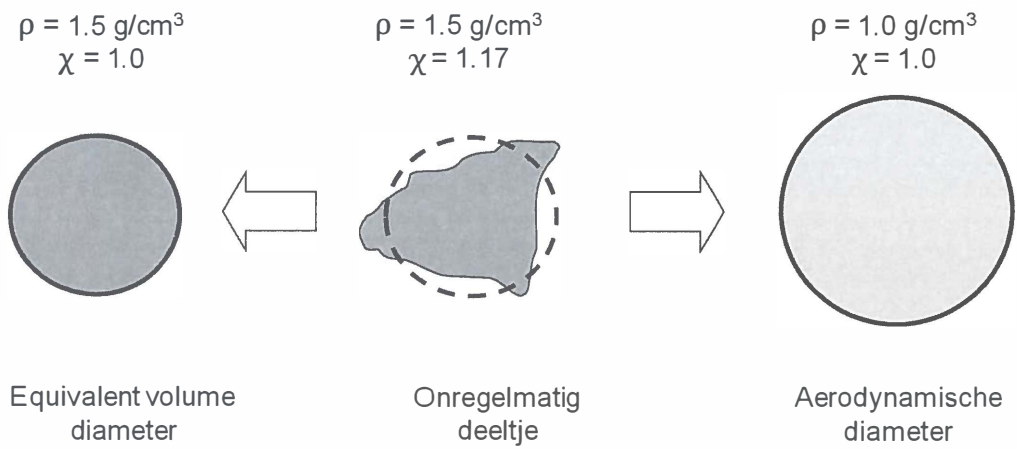
Generaties 12 -16: centrale luchtwegen
4% oppervlakte

Generaties 17-23: perifere luchtwegen
en alveoli
95% oppervlakte

Om geneesmiddelen effectief tot in de centrale en perifere luchtwegen te laten doordringen, moeten ze een deeltjesgrootte bezitten kleiner dan 5 micrometer (0.005 millimeter). Bij voorkeur moet hun diameter zelfs in het bereik tussen 1 en 3 micrometer liggen. Deeltjes groter dan 5 micrometer worden voor een belangrijk deel in mond- en keelholte en de hogere luchtwegen afgezet. Van deeltjes kleiner dan 1 micrometer wordt meestal meer dan 40 tot 50% weer uitgeademd.

Omdat deeltjes in een aerosol meestal verschillend van grootte zijn en bij droge aerosolen (vaste deeltjes) ook nog verschillende vormen en dichtheden (soortelijke gewichten) kunnen hebben, zijn de meeste aerosolen niet eenvoudig onderling te vergelijken. Het gedrag van een aerosoldeeltje wordt door al deze eigenschappen beïnvloed. De vorm kan met een (dynamische) vormfactor (χ) worden beschreven en de dichtheid (ρ) in g/cm^3. De diameter kan op verschillende manieren worden uitgedrukt. Bijvoorbeeld een

rond staafje kan zowel op lengte als op diameter worden gekarakteriseerd. Om toch een enkelvoudige uitdrukking te kunnen geven aan onregelmatig gevormde deeltjes, is in de aerosoltechnologie de equivalent volume diameter (D_E) ingevoerd. D_E is de diameter van een bol met hetzelfde volume als het onregelmatige deeltje *(Figuur 11.2)*. Deeltjes met eenzelfde equivalent volume diameter kunnen zich aerodynamisch (in lucht of in een luchtstroom) echter nog verschillend gedragen door verschillen in vorm en dichtheid. Daarom wordt in de praktijk gebruik gemaakt van de aerodynamische diameter (D_A). Een uitdrukking voor het aerodynamische gedrag van een aerosoldeeltje is de valsnelheid van dat deeltje in stilstaande lucht. D_A is daarom gedefinieerd als de diameter van een bol met de dichtheid van water die in stilstaande lucht dezelfde valsnelheid heeft als het onregelmatige deeltje. Voor een bol geldt dat de dynamische vormfactor (χ) gelijk is aan 1. De dichtheid van water is 1 g/cm³.



Figuur 2 *In de aerosoltechnologie gebruikte methoden om onregelmatig gevormde deeltjes te karakteriseren. Uitdrukking aan onregelmatige deeltjes kan worden gegeven via de equivalent volume diameter D_E, of wel de diameter van een bol met hetzelfde volume, en de aerodynamische diameter D_A, gedefinieerd als de diameter van een bol met de dichtheid van water (1 g/cm³) en dezelfde terminale valsnelheid als het beschouwde onregelmatige deeltje. Bij voorkeur wordt gebruik gemaakt van de aerodynamische diameter omdat deeltjes met gelijke D_A hetzelfde aerodynamische gedrag vertonen ongeacht hun geometrische diameter, vorm en dichtheid.* $D_A = D_E\sqrt{(\rho/\chi)}$

Aerosolen bestaande uit deeltjes van verschillende grootte zijn evenmin met een enkelvoudige diameter te beschrijven. Deeltjes van verschillende grootte kunnen in klassen worden ingedeeld op basis van aantal, volume of massa. Per diameterklasse wordt het aantal deeltjes gemeten en een aantalverdeling als functie van de diameter opgesteld. Als het aantal deeltjes per klasse in procenten van het totaal wordt uitgedrukt, wordt een frequentieverdeling verkregen. Tenslotte kan hieruit een cumulatieve frequentieverdeling worden gemaakt door de opeenvolgende klassen steeds bij elkaar op te tellen. In de aerosoltechnologie is het echter niet gebruikelijk om met aantalverdelingen te werken maar met volume- of massaverdelingen. Uit de cumulatieve verdelingen kunnen mediane diameters worden afgeleid (de 50%-waarden). Mediane diameters geven de waarde aan waarbij 50% van het totaal (in aantal, volume of massa uitgedrukt) zich in kleinere en 50% in grotere deeltjes bevindt. Bij een volumeverdeling wordt de 50% waarde als volume

mediane diameter (VMD) aangeduid, bij een massaverdeling als masa mediane diameter (MMD). Als de massaverdeling als functie van de aerodynamische diameter is gemeten, dan wordt de MMD een MMAD (massa mediane aerodynamische diameter) genoemd. In *hoofdstuk 1* wordt besproken waarom de in de literatuur veel gebruikte MMAD niet altijd de ideale variabele is om een inhalatieaerosol te beschrijven.

Voor de toediening van medicijnen in aerosolvorm aan de luchtwegen worden in de praktijk vier soorten inhalatiesystemen gebruikt. Het meest voorgeschreven zijn zogenaamde 'pressurized metered dose inhalers' (pMDI's) of dosisaerosolen. De aanduiding 'pressurised' is afgeleid van de aanwezigheid van drijfgas (gas onder druk) in de formulering. Het drijfgas zorgt door snelle verdamping voor de eigenlijke aerosolvorming uit een farmaconoplossing of –suspensie, maar is ook verantwoordelijk voor een hoge uittredesnelheid van de aerosol uit een pMDI, zoals in *hoofdstuk 1* nader wordt uitgelegd. pMDI's worden meestal gecombineerd met een voorzetkamer (valved holding chamber) of 'spacer'. Voorzetkamers worden gebruikt om vooral bij kinderen en ouderen problemen met de coördinatie tussen het afvuren van een dosis en het gelijktijdig inhaleren van de aerosol te omzeilen. Ook worden voorzetkamers gebruikt om te voorkomen dat er te veel medicijndeeltjes in de mond- en keelholte van de patiënt worden afgezet vanwege de (te) hoge snelheid waarmee het geneesmiddel uit de dosisaerosol vrijkomt. Dit heeft een verlaging van de longdosis tot gevolg en geeft kans op lokale bijwerkingen in de keel, met name bij het gebruik van inhalatiecorticosteroiden.

Een tweede type inhalatiesysteem om geneesmiddelen aan de luchtwegen toe te dienen is de droog-poederinhalator (DPI). Een droog-poederinhalator bevat geneesmiddel in de vorm van een poeder, dat door de inademingkracht van de patiënt in de juiste deeltjesgrootteverdeling moet vrijkomen. De gemicroniseerde medicijndeeltjes zijn meestal via natuurlijke adhesiekrachten aan het oppervlak van veel grotere dragerdeeltjes (lactose) gebonden en de energie aanwezig in de ingeademde luchtstroom moet de medicijndeeltjes hiervan losmaken. Als het inademen krachtiger gebeurt, leidt dat in veel gevallen tot de afgifte van kleinere geneesmiddeldeeltjes waarmee een verschuiving in de depositie naar hogere luchtwegen bij krachtiger inhaleren kan worden gecompenseerd.

Bij een derde type inhalatiesysteem wordt het geneesmiddel uit oplossing of suspensie toegediend door middel van verneveling. Een klassieke jet vernevelaar bestaat uit een spuitkop (nozzle) met twee gescheiden (co-axiale) kanalen en een persluchtsysteem. Uit het persluchtsysteem wordt lucht met hoge snelheid door een van beide kanalen geleid. Dit persluchtkanaal heeft een uitstroomopening op hetzelfde niveau als het tweede kanaal dat de farmaconoplossing aanvoert. In deze gemeenschappelijke opening wordt de farmaconoplossing door de hoge snelheid en impulsoverdracht van de luchtstroom opgebroken in een aerosol. Het precieze ontwerp van de vernevelaar en speciale voorzieningen zorgen er voor dat de juiste druppelgrootteverdeling wordt afgegeven. Modernere vernevelsystemen werken niet meer met perslucht, maar bijvoorbeeld met vibrerende membranen (vibrating mesh technology). Ze geven in combinatie met Adaptive Aerosol Delivery (AAD) alleen medicijnen af tijdens de inademing waardoor er geen aerosolverlies aan de omgeving optreedt tijdens perioden van uitademing. De daarvoor benodigde elektronica kan ook worden gebruikt om het tijdstip en de duur van toediening vast te leggen.
Tenslotte zijn er diverse nieuwe typen vernevelsystemen in ontwikkeling of reeds op de markt gebracht, zoals de Respimat®, die ook wel als Soft Mist Inhalator (SMI) wordt aangeduid.

Om in het gewenste doelgebied in de luchtwegen te komen is een combinatie van de juiste aerodynamische diameter van de geneesmiddeldeeltjes en de manier (snelheid) waarop deze deeltjes worden ingeademd van

belang. De snelheid waarmee deeltjes de luchtwegen binnen treden hangt af van de inhalatiemanoeuvre van de patiënt en de snelheid waarmee de aerosoldeeltjes door het inhalatieapparaat worden afgegeven. Meestal is de inhalatiemanoeuvre bepalend en deze hangt weer af van het gebruikte type inhalatie-apparaat. Vernevelaars geven gedurende een periode van enkele (5-15) minuten een dosis in aerosolvorm af en de patiënt neemt deze in via rustige ademhaling. De aerosol uit een vernevelaar bereikt in principe alle luchtwegen door vermenging en diffusie tijdens de langdurige uitwisseling van in- en uitgeademde lucht. De meeste andere typen inhalatoren hebben een zeer korte emissietijd waardoor de volledige dosis in één enkelvoudige ademteug wordt geïnhaleerd. Om daarmee effectief tot in de laagste luchtwegen te komen is het nodig dat de patiënt eerst diep uitademt tot restvolume en daarna diep inademt tot totale longcapaciteit. Aansluitend is het nodig om de adem gedurende tenminste 5 seconden vast te houden zodat de geneesmiddeldeeltjes voldoende tijd krijgen om uit te zakken.

Deeltjes kunnen in principe in de luchtwegen worden afgezet (en daar hun effect uitoefenen) door drie depositie-(afzettings-)mechanismen: inërtiele impactie, sedimentatie en diffusie. In de praktijk speelt diffusie echter een ondergeschikte rol van betekenis. Impactie is het botsen van een deeltje met de luchtwegwand door een (te) hoge snelheid en/of massa, waardoor het bijvoorbeeld bij een vertakking van de luchtweg in de oorspronkelijke richting blijft voortbewegen en niet de afbuiging van de luchtstroom kan volgen waarin het deeltje wordt meegevoerd. Dit principe van het 'uit de bocht vliegen' kan worden beschreven met de stopafstand van het deeltje of de op het deeltje uitgeoefende centrifugaalkracht. Om verliezen van farmacondeeltjes in de eerste bochten (mond- en keelholte) en vertakkingen van de luchtwegen via impactie gering te houden, moet de inhalatiesnelheid niet te snel zijn. Sedimentatie is het uitzakken (vallen) van een deeltje onder invloed van de zwaartekracht. Dit mechanisme van depositie wint aan betekenis als de lucht (en de daarin meegevoerde deeltjes) bijna tot stilstand is gekomen. De valsnelheid neemt toe met de tweede macht van de diameter van een deeltje. Het aantal deeltjes dat in een luchtweg kan sedimenteren is verder groter als de verblijftijd in de luchtweg langer is. Dit is dan ook de reden waarom het van belang is om de adem zo lang mogelijk vast te houden na de inhalatie van de aerosol.

Om de deeltjes in de luchtweg op de juiste plaats te krijgen is het belangrijk te weten waar het ziekteproces in de luchtwegen zich afspeelt en/of waar zich de receptoren bevinden waarop de geneesmiddelen moeten aangrijpen om hun werking te kunnen uitoefenen. De plaats van het ziekteproces en die van de receptoren bepalen het doelgebied van de medicijnen.

Bij astma is er sprake van een ontstekingsproces in de hele long, met name ook in de kleine luchtwegen. De receptoren voor inhalatiecorticosteroïden zijn ook in de hele long aanwezig, maar de dichtheid daarvan neemt toe in de richting van de kleinere luchtwegen. Daarom zijn de luchtweggeneraties 7 tot 23 het belangrijkste doelgebied voor inhalatiecorticosteroïden, die nog steeds de beste onderhoudsbehandeling geven tegen ontstekingen bij astma *(Hoofdstuk 2)* en COPD. Luchtwegverwijders bij astma en COPD ontspannen de spieren om de luchtwegen waardoor deze niet meer worden vernauwd. Deze spieren zijn vooral om de grotere en middelgrote luchtwegen aanwezig. De luchtwegen met spieren zonder kraakbeen vormen de generaties 7-14. Het doelgebied voor luchtwegverwijders ligt dus wat hoger dan dat voor inhalatiecorticosteroïden (generatie 7-23).

Bij Cystic Fibrosis zijn de bacteriën die met antibiotica moeten worden behandeld ook in de hele luchtweg aanwezig, evenals het slijm waarvoor slijmverdunners kunnen worden geïnhaleerd.

Geneesmiddelen die in de bloedbaan terecht moeten komen, moeten voornamelijk via de longblaasjes worden geabsorbeerd en dus heel diep worden afgezet. Een voorbeeld hiervan is insuline *(Hoofdstuk 8)*.

In hoofdstuk 2 wordt de huidige onderhoudsbehandeling van astma besproken. Inhalatiecorticosteroïden (ICS) vormen daarbij nog steeds de hoeksteen. Uit het feit dat het ontstekingsproces van astma in de hele luchtweg aanwezig is tot in de hele kleine luchtwegen en dat in die kleinere luchtwegen bovendien het grootste aantal van de ICS-receptoren aanwezig is, evenals de grootste fractie van het totale luchtwegoppervlak, kan worden geconcludeerd dat in de perifere luchtwegen ook de grootste fractie van de totale dosis gewenst is om de ontsteking zo goed mogelijk te kunnen behandelen.

In hoofdstuk 3 worden de deeltjesgrootteverdelingen van de aerosolen uit de in Nederland meest gebruikte ICS-pMDI's met elkaar vergeleken. De resultaten laten zien dat de op het drijfgas HFA gebaseerde pMDIs alleen een fijnere aerosol afleveren (t.o.v. de oudere CFK-pMDI's) als zich als oplosmiddel ethanol in de samenstelling bevindt. Overigens garandeert een fijnere aerosol (kleinere MMAD) niet altijd een grotere fractie (hoeveelheid) in het voor ICS relevante deeltjesgroottebereik van 1 tot 3 micron, doordat deze fijnere aerosolen ook meer submicron deeltjes (< 1 µm) bevatten. Een opvallende bevinding is verder dat van het veel gebruikte fluticason in de sterktes 250 en 125 µg, de grootte van de afgeleverde dosis afneemt met het aantal afgegeven doses. Deze studie toont aan dat er een groot verschil is in de fijne deeltjes fractie van verschillende merken pMDIs, waarbij de fijne aerosolen vanwege hun grote fijne deeltjes fractie in het gewenste bereik het meest geschikt lijken voor kinderen.

Hoofdstuk 4 behandelt de variabelen die een invloed uitoefenen op de afgeleverde (fijne deeltjes) dosis uit een voorzetkamer. Het doel van het onderzoek in dit hoofdstuk was om te onderzoeken wat de dosis uit een voorzetkamer is ten opzichte van de dosis uit de pMDI waarmee de voorzetkamer wordt gecombineerd en welke combinatie van voorzetkamer en pMDI uit de beschikbare mogelijkheden de grootste fijne deeltjesfractie levert. De invloed van verschillende variabelen, zoals de snelheid van inademen vanuit de voorzetkamer, de tijdsduur van wachten tussen afvuren van een dosis in de voorzetkamer en het inhaleren daarvan en de relatieve luchtvochtigheid is hierbij onderzocht. In de literatuur wordt vaak aangegeven dat voorzetkamers de beschikbare longdosis kunnen verhogen door het selectief achterhouden van grotere deeltjes (die door de zwaartekracht in de voorzetkamer zouden neerslaan). Voor de in *hoofdstuk 4* onderzochte combinaties is het selectief uitzakken van de grotere aerosoldeeltjes echter niet aangetoond. Er is verder geen effect gevonden van de inhalatiekracht waarmee de aerosol uit de voorzetkamer wordt geïnhaleerd op de deeltjesgrootteverdeling van de aerosol. Te snel inademen is echter niet goed voor de longdepositie omdat het leidt tot meer keeldepositie. Het effect van tot 20 seconden wachten tussen het afvuren en inhaleren van een dosis is veel geringer dan in eerdere, beperktere, studies werd vastgesteld. De meest interessante bevinding uit deze studie is dat een verhoging van de luchtvochtigheid kan resulteren in een verhoging van de voorzetkameroutput met maximaal een factor 2. Naar aanleiding van deze studie zou een praktisch advies kunnen zijn om een ICS-pMDI met voorzetkamer in een pas gebruikte badkamer te gebruiken. De toename in output bij verhogen van luchtvochtigheid heeft geen effect op de deeltjesgrootteverdeling in de aerosol. Een verhoging van de luchtvochtigheid resulteert dus direct in een grotere kleine deeltjesfractie.

In hoofdstuk 5 wordt uitgelegd wat het doelgebied is voor inhalatie-antibiotica bij CF en met welke inhalatiemanoeuvre en welk type toedieningsvorm dit doelgebied het beste te bereiken is. De luchtwegschade, de infecties en ontstekingen bij CF bevinden zich in alle luchtwegen, van groot tot klein. De gehele long is daarom het doelgebied. Vanwege het hiervoor aangegeven enorme verschil in oppervlakte tussen de hogere plus centrale luchtwegen, die samen slechts 5% van het luchtwegoppervlak bedragen, en de perifere luchtwegen die 95% aan het totale oppervlak bijdragen *(Figuur 1)*, is het het beste om de depositie te richten op die perifere luchtwegen. De grote luchtwegen worden daarbij voldoende behandeld met de verliezen die met name door impactie onderweg in de hogere en centrale luchtwegen optreden. Ondanks perifere 'targeting' zal het concentratieverschil voor het medicijn tussen de hogere en centrale ten opzichte van de perifere luchtwegen groot zijn en tenminste een factor 25 bedragen!

Hoofdstuk 6 beschrijft het effect van het dagelijkse gebruik van twee typen vernevelsystemen voor de inhalatie van antibiotica (voor een periode van 6 maanden) door volwassenen met CF op het functioneren van deze systemen. In de praktijk is dit effect afhankelijk van het opvolgen van de reinigingsadviezen. Vernevelsystemen die bestaan uit een vernevelaar en een compressor zijn vaak volumineus en lawaaiig. Toen een stil, klein vernevelapparaat beschikbaar kwam dat op batterijen werkte, werd dat al snel enorm populair. Deze studie laat zien dat het nieuwe, stillere en snellere systeem, de eFlow rapid® druppeltjes produceert die met een VMD van 3,5 µm aanzienlijk groter zijn dan de 2,8 µm van de geregistreerde Pari LC PLUS® vernevelaar met TurboBoy N® compressor. Doordat de kans op impactie van een deeltje toeneemt met het kwadraat van de diameter, is de kans op mond- en keelverliezen voor de aerosol uit de eFlow rapid® bij een gelijke inhalatiemanoeuvre dus aanzienlijk groter dan die voor de aerosol uit de Pari LC PLUS®. Gebleken is dat van de aerosolen uit beide systemen de gemiddelde deeltjesgrootte na 6 maanden gebruik nog verder toeneemt waardoor beide aerosolen ook gedurende de tijd minder geschikt worden voor depositie in de kleine luchtwegen. De eFlow rapid® maakt gebruik van een zogenaamde 'vibrating mesh', een geperforeerd membraan dat aerosoldruppeltjes in de gewenste grootte afgeeft. Uit de studie werd duidelijk dat patiënten ondanks het advies om dit niet te doen, hetzelfde systeem ook voor andere geneesmiddelen gebruikten en bovendien de mesh niet volgens de instructies schoonmaakten. Dit leidde tot een vermindering van de output per minuut door verstopping van een deel van de gaatjes in de mesh, en omdat de eFlow rapid® oorspronkelijk na 10 minuten automatisch afsloeg, tot een vermindering van de toegediende dosis. Na verwisseling van de mesh functioneert het apparaat weer naar behoren. Uit deze studie blijkt ook dat communicatie tussen zorgverlener en patiënt heel belangrijk is en toch vaak niet leidt tot het juist opvolgen van adviezen.

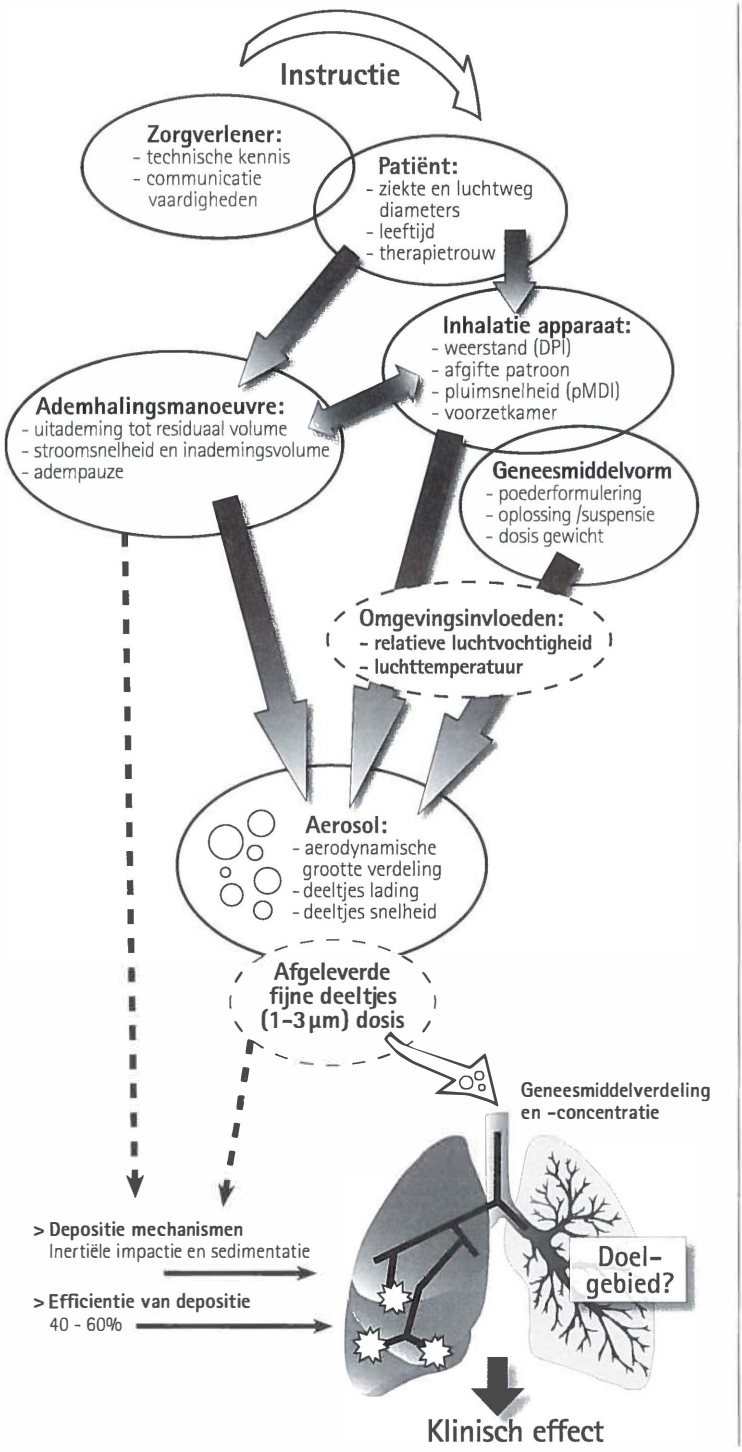
Hoofdstuk 7. Twee antibiotica, tobramycine en colistine, die bij CF veel gebruikt worden zijn elk in twee verschillende vernevelvormen (formuleringen) beschikbaar. Deze vier variaties werden op deeltjesgrootteverdeling onderzocht uit vier verschillende vernevelaars. De vernevelaars werden elk getest met 4 verschillende verneveldrukken, omdat bekend is dat een hogere druk leidt tot een kleinere deeltjesgrootte. Onder dezelfde omstandigheden getest (dezelfde vernevelaar met dezelfde verneveldruk) leveren alle vier formuleringen dezelfde deeltjesgrootteverdeling op en daarom zijn ze vanuit dat oogpunt onderling uitwisselbaar. De vernevelaars zijn dat zeker niet. Bij 1 bar varieert de VMD van 3.5 tot 2.5 µm; bij 2,5 bar is dit verschil kleiner: de VMD varieert dan tussen 2,2 en 1,6 µm. De Ventstream® vernevelaar blijkt de kleinste VMD te leveren onder alle omstandigheden en is daarmee het meest geschikt voor een hoge depositie in de perifere luchtwegen en evenredige verdeling over de hele luchtweg met de juiste ademhalingsmanoeuvre.

In hoofdstuk 8 is een studie beschreven waarin met een destijds beschikbare (inhaleerbare) droog poeder formulering van insuline (Exubera®) een patiënt werd behandeld met diabetes. Eerst werd bepaald wat de primaire deeltjesgrootteverdeling is van deze insulinevorm (door middel van laser diffractietechniek uit RODOS dispersie). Daarna is gekeken wat de massafractie aan kleine deeltjes (1-3 micron) is van deze insulinevorm vanuit twee verschillende inhalatoren, de Exubera® inhalator en de Twincer™. Ook is gekeken naar het effect van de doseringen uit deze inhalatoren op de deeltjesgrootteverdelingen van de afgeleverde aerosolen. Op basis van de verschillen in in vitro depositie werd de hoeveelheid insuline bepaald die via beide inhalatoren moet worden toegediend voor eenzelfde klinische effect (dezelfde insuline spiegel). Daarbij is gebleken dat de Twincer™ met de helft van de Exubera® insulineformulering equivalent is aan de Exubera® inhalator. Het unieke van deze studie is dat hiermee is aangetoond dat de combinatie van een hoge fijne deeltjesfractie met de juiste inhalatie-instructie en inhalatiemanoeuvre inderdaad leidt tot meer opname van insuline in het lichaam. De Twincer™ geeft daarnaast de volledige dosis af in de eerste fase van de inademing, waardoor het medicijn ook tot in de diepste luchtwegen kan doordringen. Al deze verbeteringen ten opzichte van de Exubera® inhalator bieden goede perspectieven voor de terugkeer van inhaleerbare insuline, maar belangrijker nog, het biedt ook goede mogelijkheden om middelen tegen bijvoorbeeld tuberculose, longkanker en vaccins (zoals tegen mazelen) via de luchtwegen toe te dienen.

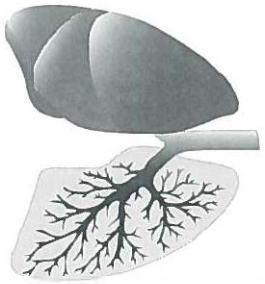
In hoofdstuk 9 worden de variabelen die de effectiviteit van inhalatietherapie bepalen in hun onderlinge samenhang aan de hand van een figuur *(Figuur 3, pagina 166)* besproken.

Samenvattend is het duidelijk dat de keuze van de toedieningsvorm (de inhalator), inclusief de keuze voor een specifieke voorzetkamer bij een bepaald type pMDI, de afgeleverde fijne-deeltjesfractie bepaalt. Externe factoren, zoals de luchtvochtigheid, kunnen hierbij van belang zijn: een hogere luchtvochtigheid is gunstig voor de fijne-deeltjesfractie uit een voorzetkamer, maar ongunstig voor sommige droog-poederinhalatoren, omdat achtergebleven poederresten aan de inhalatorwanden kunnen plakken. De communicatie en interactie tussen zorgverlener en zorggebruiker is van groot belang voor juiste voorbereidingshandelingen en inhalatietechniek, maar ook het opvolgen van bijvoorbeeld schoonmaakinstructies. Er zijn dus nog veel verbeteringen mogelijk. Daarvoor worden in dit hoofdstuk 9 verdere aanbevelingen gedaan.

Tot slot is communicatie ook bij samenwerken en het schrijven en beoordelen van medisch onderzoek van belang. In de appendix staat beschreven hoe cultuurverschillen tussen Engelsen en Nederlanders ondanks een gemeenschappelijke taaloorsprong en veel andere parallellen, toch tot een andere interpretatie van Engelse uitspraken kunnen leiden.



Figuur 3
De output uit inhalatie toedieningssystemen is van vele factoren afhankelijk.



DANKWOORD

DANKWOORD

Heel veel mensen hebben een bijdrage geleverd aan mijn ontwikkeling, en daarmee ook aan de totstandkoming van dit proefschrift. Het dankwoord biedt een uitgelezen gelegenheid hen daarvoor te bedanken! De opdracht is niet voor niets "To all who teach".

Mijn promotor, prof. dr. Eric Duiverman, bij de kennismaking in je huis in Moordrecht klikte het meteen en ik werd kinderlongarts in opleiding in Groningen. Ik ben je enorm dankbaar voor het altijd in mij gestelde vertrouwen en de ruimte die je altijd biedt. Ik bewonder hoe goed je beslissingen kunt nemen en de belangrijke vertrouwensrol die je voor veel mensen in het kinderziekenhuis hebt. Je hebt onze afdeling gemaakt tot een hele krachtige, met eenheid in verscheidenheid.

Mijn co-promotor, dr. Anne de Boer, de Willy Wortel van de afdeling Farmaceutische Technologie en Biofarmacie, door je snelle denken en je tomeloze enthousiasme duizelde het soms als ik bij je vandaan kwam, maar je zekerde hiermee de kwaliteit van dit proefschrift. Bedankt voor al je praktische en morele steun.

De leden van de beoordelingscommissie, prof. dr. Dirkje Postma, prof. dr. Paolo Colombo en prof. dr. Rolf Berger, wil ik alle drie bedanken voor het beoordelen van het manuscript. Prof. Colombo, although we have not yet met in person, it immediately became clear to me that you are a fast working, very friendly and charming authority in the field. I am very pleased that you will be attending the defence of my thesis. Prof. dr Berger, kindercardioloog, wij delen niet alleen de belangstelling voor de organen in de borstkas (ook al zijn we het soms oneens of het 'cardiaal'of 'pulmonaal' is), we hebben ook veel raakvlakken op het gebied van de patiëntenzorg en transplantatie, van met name kinderen met pulmonale hypertensie. Vanwege deze raakvlakken, je leuke collega's en je positieve uitstraling ben ik heel blij dat je lid bent van de beoordelingscommissie.

Prof. dr. Dirkje Postma voor jou begin ik een nieuwe alinea. Ik wil je bedanken voor je enorme morele steun en praktische hulp bij een aantal manuscripten. Manuscripten die daardoor beknopter en veel beter leesbaar werden. Ik vind het enorm jammer dat je er voor hebt gekozen toch naar Australië te gaan, maar ben blij dat dr. Nick ten Hacken wel beschikbaar is voor de 'grote commissie', ook als gewaardeerd vertegenwoordiger van het GRIAC: het gerenommeerde Groningen Research Institute for Asthma and COPD.

Prof. dr. Henk Jan Verkade, jij wilde mij 'even' spreken eind 2011 en daarmee volgde een periode van 'overview first, then details', met zowel richting als perspectief. Dank je wel voor de wending die je daarmee bereikt hebt, het enthousiasme en de (tijds)inzet waarmee je hebt gespiegeld. Ik hoop dat je snelle denken gepaard aan de vraag 'of ga ik soms te langzaam' niet tot problemen voor mij leidt bij de verdediging.

Prof. dr. Pieter Sauer, de eerste maanden in Groningen ben je zelfs nog mijn opleider tot kinderarts geweest. In de loop der jaren is er een vriendschap ontstaan, met als kroon ons enthousiasme voor de stedenband Groningen-Murmansk, waarbij we interactief kindergeneeskundig onderwijs in St. Petersburg hebben kunnen lanceren. Ik ben heel blij dat je op 28 november de voorzitter van de promotiecommissie kunt zijn.

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Vries, kinderarts in Leeuwarden, jou ken ik door onderwijs, patiëntenzorg en dit onderzoek, vol ideeën voor onderzoek en met oog op praktische toepasbaarheid, morele ondersteuner. Gianni Bocca, nog meer Italiaanse invloed in het onderzoek, dank je wel voor jouw steun en vriendschap en dat je mijn grappen over Italianen zo weet te waarderen en te pareren. Jet van der Hulst delen we nog, maar gelukkig hoort Marianne Zwolsman bij het CF-team en niet bij het diabetes team. Anne Lexmond, jij hebt al een start gemaakt met inspirerend vervolgonderzoek.

Mijn directe collega´s prof. dr. Gerard Koppelman, dr. Elianne Vrijlandt en dr. Brigitte Willemse (´de fellow´, maar niet zomaar één) die samen met onze kinderallergoloog prof. dr. Ewoud Dubois en geleid door prof. dr. Eric Duiverman onze sectie vormen, hartelijk dank voor jullie collegialiteit en steun. Wat fijn dat jullie mij veel ruimte gaven voor het schrijven van de laatste stukken voor dit proefschrift. Dr. Jorrit Gerritsen, jij hebt mij en mijn gezin altijd heel hartelijk ontvangen en ook als kinderlongarts van het eerste uur bijgedragen aan mijn opleiding, aangevuld met ERS (European Respiratory Society) perspectief.

Het werk in een academisch kinderziekenhuis is zo boeiend door het samenwerken, opleiden en de multidisciplinaire problematiek. De kinder-thoraxchirurgen prof. dr. Tjark Ebels en Tjalling Waterbolk, bedankt voor open overleg rond congenitale longafwijkingen en prachtige transplantaties. Nu maken ook Sarah Arrigoni, Yvonne den Drijver en dr. Guido Michielon deel uit van het kinderteam. De transplantatie artsen dr. Wim van der Bij, dr. George Nossent en dr. Erik Verschuuren, bedankt dat ik jullie voor een donor aanbod en transplantatie overleg altijd lastig kan vallen. Dr. Michiel Erasmus, transplantatiechirurg en Willie Steenhuis als boegbeeld van het secretariaat longtransplantatie, ook hartelijk dank. Verder mijn kamergenoot dr.Terry Derks, de kinderintensivisten en alle andere (deelspecialistische) collega´s, verpleegkundigen en secretaresses van het kinderziekenhuis die zich zo inzetten. Anneloes, jij figureert ook als kinderarts in opleiding in dit boekje, dank je wel. Een belangrijk deel van het werk heeft betrekking op CF: bedankt collega´s van het CF team, ook het volwassen CF team voor de goede samenwerking. Marjanne van Smaalen, longfunctie, bedankt ook voor plannen van kinderen voor de virtuele astmakliniek samen met Christine van Baak en het secretariaat van Jackeline van Gelder en Aaf Teuben.

De basis voor de belangstelling voor kinderlongziekten is gelegd in het WKZ, door dr. Hein Brackel en dr. Han van der Laag. Prof. dr. Kors van der Ent, dank je wel voor de weloverwogen gesprekken over kindergeneeskunde en kinderlongziekten in het laatste deel van mijn opleiding. Dank je wel dat je wil deelnemen aan de oppositie. Natuurlijk hebben met name de kinderen met CF, die in de jaren 90 nog vaak bij elkaar lagen (op afdeling Eskimo) en altijd genoeg catering hadden en verhalen om de diensturen goed door te komen, mijn enthousiasme in de richting van kinderlongziekten laten gaan.

Dr. Christian Benden, thank you for introducing me in the International Pediatric Lung Transplantation Consortium (IPLTC), with dr. Sam Goldfarb as a chair and dr. Jackson Wong as treasurer, all making me feel very welcome. Prof. dr. Ernst Eber chair of the Pediatric Assembly (ERS), thank you for allowing me to serve as a secretary for the Paediatric Asthma and Allergy group. Prof. dr. Andy Bush, thank you for speaking your famous words (meant for another collegue) "May his problems be huge, and his fellow unwell" as well as our mutual efforts to improve British-Dutch understanding as described in this thesis.

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hebben gehad in Amsterdam is heel bijzonder. Lex Verstraaten, jou wil ik graag bedanken voor alle ruimte die je me binnen NW gegeven hebt! Het werk als scheepsarts bij de Koninklijke Marine, volgend op de militaire vorming in Bak 93-2, heeft me ook een nieuwe wereld laten kennen. Wat een bemanning, speciale dank aan mijn commandant Rob Teeuwisse*, eerste officier Huibert van Eijsden en het hoofd logistieke dienst Frank Marcus.

Al meer dan 10 jaar WinterKLAS organisatie met Károly Illy en prof. dr. Paul Brand. De Werkgroep Inhalatietechnologie, met nu onder andere prof. dr. Richard Dekhuijzen (of zoals de gestrande terugreis vanuit Ierland duidelijk maakte mr. iPhone), prof.dr. Wim van Alderen en mijn bunkmaatje dr. Hans in 't Veen. Het redigeren van het werkboek kinderlongziekten, geïnspireerd door Lucky Luke en de Daltons, geleid door MA, was heel leuk en heeft me veel over formuleren en schrijven geleerd. Enorm bedankt daarvoor, dr. Mariëlle Pijnenburg, dr. Peter Merkus en dr. René van Gent. Het is heel bijzonder dat november 2012 ook dit boek oplevert. De basis daarvoor werd gelegd op een vergadering met één tapa per persoon. Peter, ik hoop dat je je als Dalton gepast kleedt als lid van de oppositie.

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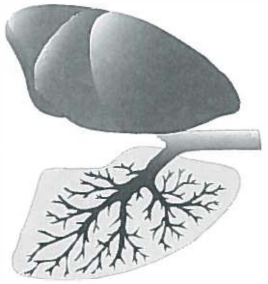
Twee weken als coassistent bij dr. Andrew Ratchford*, zijn vrouw Jackie en hun drie dochters waren genoeg voor Jackie om bij het afscheid te zeggen: "I'm sure Andrea deserves a medal for putting up with you". Ik kan niets anders dan dat toegeven: Lieve Andrea, bij ons trouwen heb je die gouden medaille van hen ook gekregen en je verdient hem nog steeds dubbel en dwars!

Bart Rottier, oktober 2012



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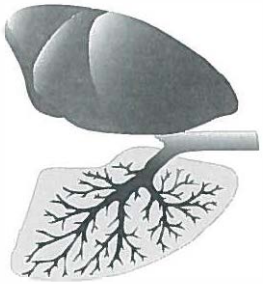
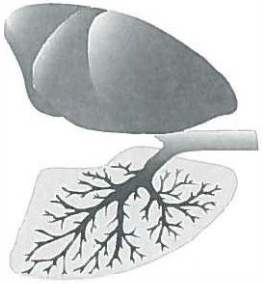


ABBREVIATIONS

ABBREVIATIONS

ABPA	Allergic bronchopulmonary aspergillosis
AC	AeroChamber®
BDP	Beclomethasone diproprionate
BPD	Bronchopulmonary dysplasia
BUD	Budesonide
CAI	Cascade impaction analysis
CF	Cystic fibrosis
CFC	Chlorofluorocarbon
CIC	Ciclesonide
CLD	Chronic lung disease
C_{max}	Maximal concentration
COPD	Chronic obstructive pulmonary disease
C_{opt}	Optical concentration in the aerosol.
CT	Computed tomography
DA	Aerodynamic diameter
DE	Equivalent volume diameter
DPI	Dry powder inhaler
FEV_1	Forced expiratory volume in 1 second
FPD	Fine particle dose
FP	Fluticasone diproprionate
FPF	Fine particle fraction
FRC	Functional residual capacity
GSD	Geometric standard deviation (for log-normal distributions)
HFA	Hydrofluoralkane
ICS	Inhaled corticosteroids
LDA	Laser diffraction analysis
MIC	Minimal inhibitory concentration
MMD	Mass median diameter
MMAD	Mass median aerodynamic diameter
MRI	Magnetic resonance imaging
NEB	Nebuhaler®
PA	*Pseudomonas aeruginosa*
PCD	Primary ciliary dyskinesia
pMDI	Pressurized metered dose inhaler
PSD	Particle size distribution
RCT	Randomized controlled trial
RH	Relative air humidity
RS	Relative span of the size distribution; ratio of $(X_{90}–X_{10})$ to X_{50}
T1DM	Type 1 diabetes mellitus
TBC	Tuberculosis
TIP	Tobramicin Inhalation Powder
TLC	Total lung capacity
VHC	Valved holding chamber
VMD	Volume median diameter
VOL	Volumatic®
X_{10}	Diameter derived from the cumulative volume distribution curve: 10% of the volume is in particles smaller than X_{10} (micron)
X_{50}	Diameter derived from the cumulative volume distribution curve: 50% of the volume is in particles smaller than X_{50} (micron)
X_{90}	Diameter derived from the cumulative volume distribution curve: 90% of the volume is in particles smaller than X_{90} (micron)



CURRICULUM VITAE

Bart Rottier was born in Amsterdam on august 6th 1965. He attended the Petrus Canisius College in Alkmaar and graduated from gymnasium-ß in 1984. He then enrolled in medical school and graduated as a medical doctor (with honours) from the Free University, Amsterdam, in 1992.
Extra curricular activities included two summers as a camp counselor in Camp Poyntelle, Pennsylvania (1987 and 1988), and a clinical clerkship in general practice on the Isle of Lewis, Outer Hebrides (dr. Ratchford and family), followed by a job as inflight interpreter with Northwest Airlines (1989).
After the military service as a ship's doctor (HNLMS Willem van der Zaan, 1993), Bart was trained to become a pediatrician from 1994 onwards in the Wilhelmina Children's Hospital, Utrecht (head: prof dr. J.W. Stoop and prof. dr. J.L.L. Kimpen) and the St Elisabeth Hospital, Tilburg (dr. J. Draaisma). His last active naval duty was giving pediatric input to develop a treatment kit used in the Dutch Role 2 hospital in Afghanistan (2008).

In 1999 he started his clinical fellowship in pediatric pulmonology at the University Medical Center Groningen under supervision of prof. dr. E.J. Duiverman, head of the division. Bart qualified as a consultant in pediatric pulmonology in 2002. He is an active member of the Medical Advisory Board of the Dutch Cystic Fibrosis Society (NCFS) and the International Pediatric Lung Transplantation Consortium (IPLTC). In 2006 he started the research for this thesis at the Department of Pharmaceutical Technology and Biopharmacy (head: prof. dr. H.W. Frijlink).

He is co-author of a book on pediatric pulmonology for general practitioners and pediatricians in training ("Praktische Kindergeneeskunde: kinderlongziekten", 2002) and in 2012 he finished co-editing an update of a practical book on pediatric pulmonology ("Werkboek Kinderlongziekten").

Teaching activities include co-organizing the yearly WinterKLAS symposium, a case based, problem oriented course on pediatric pulmonology, allergology and communication in Davos.
He is a licensed APLS (Advanced Pediatric Life Support) instructor and a trainer in Motivational Interviewing. He actively participates in a city twinning (Groningen-Murmansk) project headed by prof. dr. P.J.J. Sauer on improving pediatric care in St. Petersburg and Murmansk by participating in workshops on pediatric pulmonology, pediatric intensive care and neonatology, as well as interactive teaching methods and literature searching.

He was a member of the Scientific Committee of the Dutch Pediatric Society (2004-2010). In 2011, he was elected secretary for the Paediatric Asthma and Allergy group of the European Respiratory Society.

Bart is married to Andrea Rottier-de Gier, who is a biology teacher and together they have 3 sons: Maarten (1996), Pepijn (1998) and Sebastiaan (2000). They participate on high level in the Dutch swimming league and complete the factors that lead to a lack of sleep.